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Biological Invasions: Biogeography and Multitrophic Interactions

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BIOLOGICAL INVASIONS: BIOGEOGRAPHY AND MULTITROPHIC INTERACTIONS

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ABSTRACT

Species interactions play a prominent role in the establishment and spread of many invasive species. However, rarely are invasions studied in more than a direct pairwise species context, or with consideration to how species interactions can vary biogeographically. Using field surveys combined with common garden and greenhouse experiments, I investigated how multitrophic above and belowground interactions influence plant invasions at large spatial scales. I focused on comparisons between sympatric native and invasive lineages of *Phragmites australis*, a wetland grass distributed throughout North America.

I conducted a field survey to examine support for the enemy release hypothesis in a tritrophic framework. In North America, the invasive lineage of *P. australis* escaped from introduced *Lipara* gall-flies, attributed to greater vertebrate predation on *Lipara* infesting the invasive than the native lineage. A complementary common garden experiment revealed that enemy release of the invasive *P. australis* lineage from *Lipara* was driven by local environmental conditions rather than genetic differences between the two lineages. Importantly, local enemy release was strongest at northern latitudes, generated by genetically based non-parallel latitudinal gradients in *Lipara* herbivory for the native and invasive lineages. This phenomenon could translate to biogeographic variation in invasion success and is worthy of investigation across a range of invaded systems and species interactions.

I also conducted a greenhouse experiment to examine the interactive effects of rhizosphere soil biota, interspecific competition, and nutrient availability on performance of *P. australis* and native smooth cordgrass, *Spartina alterniflora*. All lineages of *P. australis* suffered negative impacts from soil biota, suggesting this interaction does not directly facilitate the success of invasive *P. australis*. However, the most interesting result from this experiment was

that soil biota from the invasive *P. australis* lineage negatively impacted *S. alterniflora*, whereas soil biota from the native lineage had a positive impact. This indirect spillover of pathogens and mutualists interaction may have important implications for invasion success and restoration. In summary, my dissertation highlights the importance of examining biological invasions in a biogeographic and multitrophic context and has broad implications for the understanding and management of biological invasions.

CHAPTER 1
INTRODUCTION

IMPACTS AND CAUSES OF BIOLOGICAL INVASIONS

In recent decades, expanding human migration, transport and trade has resulted in both incidental and intentional redistribution of a diverse array of species to novel ecosystems around the globe (Levine and D'Antonio 2003; Hulme 2009). Few of these introduced species survive the journey or the multitude of novel biotic and abiotic factors in the introduced range (Mack et al. 2000). However, inevitably a proportion will establish, persist and proliferate, ultimately becoming invasive (Richardson et al. 2000a), with potential to inflict devastating ecological and socioeconomic consequences. The ecological impacts of invasive species are diverse and include biodiversity loss, shifts in evolutionary pathways, the vectoring of diseases, and alteration of ecosystem processes such as fire regimes, hydrology and nutrient cycling (Vitousek et al. 1995; Mack et al. 2000; Mooney and Cleland 2001; Vila et al. 2011). For example, the invasive plant alligator weed (*Alternanthera philoxeroides*) can produce dense mats in littoral and terrestrial habitats, displacing flora and fauna, altering water flow and quality, disrupting nutrient regimes, providing habitat for disease-carrying mosquitos, and degrading pasture, turf and crop production (Sainty et al. 1998; Pan et al. 2007). There are more than 50,000 invasive species in the United States and the economic cost associated with them is estimated at over $120 billion annually (Pimentel et al. 2005), while worldwide losses to invasive species are estimated at around 5% of the global economy (Pimentel et al. 2001). For example, management of diamondback moth (*Plutella xylostella*) alone costs an estimated $5 billion per year to growers of cruciferous vegetable crops (e.g., broccoli, cabbage, kale, mustard, radish, watercress) around the world (Zalucki et al. 2012).

Increased recognition of the substantial problems posed by invasive species has resulted in a dramatic expansion in the biological invasions literature over the last two decades (Lowry et al. 2013). One broad question which has received a strong research focus but remains unanswered is "why do some introduced species become invasive whereas others fail to establish or remain relatively benign (i.e., naturalized species)?" Investigating this question enables better understanding of mechanisms underpinning the colonization and spread of invasive species, which is critical to predicting and preventing future invasions, as well as managing established invaders. Moreover, studying biological invasions also provides an unfortunate yet profitable opportunity to further our knowledge of fundamental ecological concepts, largely due to the parallels between many invasion and general ecological hypotheses (i.e., the biotic resistance and diversity-stability hypotheses) (Elton 1958; Shea and Chesson 2002; Ives and Carpenter 2007; Jeschke 2014).

The competing hypotheses and sub-hypotheses proposed to explain the causes of biological invasions (e.g., Catford et al. 2009; Jeschke et al. 2012) are almost as diverse and interrelated as the impacts of invaders, and it is clear there is no "silver bullet" hypothesis that can elucidate the underlying basis of all invasions. Some factors which have consistently been demonstrated as important drivers of invasions include natural and anthropogenic habitat disturbance/alteration (Hobbs and Huenneke 1992; D'Antonio et al. 1999; Bhattarai and Cronin 2014), propagule pressure (Lockwood et al. 2005; Colautti et al. 2006), and environmental matching (Peterson 2003; Jiménez-Valverde et al. 2011). However, another suite of hypotheses that has received considerable attention is the influence of species interactions (e.g., competition, herbivory, predation/parasitism, mutualisms), which have emerged as highly influential in determining the success of introduced species as well as the resistance/susceptibility of native

communities to invasion. For example, invasive plant species are often successful due to possessing stronger interspecific competitive ability for resources than co-occurring native species (e.g., Elton 1958, Bakker and Wilson 2001; Vila and Weiner 2004; Gioria and Osborne 2014). The enemy release hypothesis (Elton 1958; Keane and Crawley 2002) is also broadly supported in the literature (e.g., Wolfe 2002; Mitchell and Power 2003; Liu and Stiling 2006) and posits that invasive species leave behind natural enemies from their native range, enabling proliferation in the introduced range. Closely intertwined with interspecific competitive ability and the enemy release hypothesis are the concepts of biotic resistance (Elton 1958) and local enemy release (Zheng et al. 2012). Biotic resistance arises when native competitors and/or natural enemies present in the introduced range impede invasive species more strongly relative to co-occurring native species (e.g., Maron and Vila 2001; Agrawal and Kotanen 2003; Levine et al. 2004; Parker and Hay 2005; Chun et al. 2010; Morrison and Hay 2011; Fan et al. 2013). Conversely, local enemy release (or biotic susceptibility) would be represented by invasive species suffering less damage from competitors and/or natural enemies than native species (e.g., Dietz et al. 2004; Agrawal et al. 2005; Parker and Gilbert 2007; Funk and Throop 2010; Zheng et al. 2012). Finally, beneficial interactions of invasive species with native and co-introduced soil biota (e.g., Parker 2001; Pringle et al. 2009; Dickie et al. 2010; Klock et al. 2015), pollinators (e.g., Barthell et al. 2001; Geerts and Pauw 2009), dispersers (e.g., Pearson and Ortega 2002; Gosper et al. 2005), and other mutualists (e.g., Helms 2013) also play a vital role in many biological invasions (reviewed by Richardson et al. 2000b; Traveset and Richardson 2014).

While it is clear that direct species interactions can be important to invasion success, invasive species interact directly and indirectly (e.g., trophic cascades, apparent competition, intraguild predation) with a complex community of organisms over multiple trophic levels (Holt

1977; Strauss 1991; Wootton 1994; Pace et al. 1999; Walsh 2013); yet, invasions are rarely studied in more than a direct pairwise species context. For example, the influence of higher trophic levels (i.e., predators and parasitoids) has largely been ignored by invasion biologists investigating enemy release of invasive plants (Harvey et al. 2010, but see Engelkes et al. 2012; Kim et al. 2014). Moreover, multiple introduced species may facilitate one another's spread or act synergistically to worsen their impact on native species, a process termed invasional meltdown (Simberloff and Von Holle 1999). Such complex multitrophic and indirect interactions are only likely to become more common as invasive species become more prevalent and interact more frequently, and their potential role in facilitating and preventing invasions is in urgent need of investigation.

Another inherent quality of many invasions is that they often occur over broad spatial scales (i.e., entire continents) and thus interact with large-scale ecological and evolutionary processes. Consequently, biogeographic approaches are increasingly being applied to invasion research (e.g., Colautti et al. 2014; Cronin et al. 2015). A particularly relevant biogeographic prediction in ecology is that the strength of species interactions involving native species should evolve to exhibit a latitudinal gradient (Dobzhansky 1950; Coley and Aide 1991; Schemske et al. 2009, but see Moles et al. 2011). Conversely, invasive species may not exhibit a parallel latitudinal gradient due to having insufficient time to evolve or responding differently to selection pressures. Such a pattern can have important implications for invasion success. For example, if sympatric native and invasive plant species exhibit dissimilar latitudinal gradients in response to their natural enemies, competitors and/or mutualists, this could lead to heterogeneity in community resistance/susceptibility at a biogeographic scale (Fig. 1.1) (Bezemer et al. 2014; Cronin et al. 2015). Recent studies have demonstrated non-parallel gradients between native and

invasive taxa may be common (e.g., Cronin et al. 2015; Bhattarai et al. in review), although the proximal mechanisms underlying differences in the direction and strength of latitudinal gradients are still relatively unexplored.

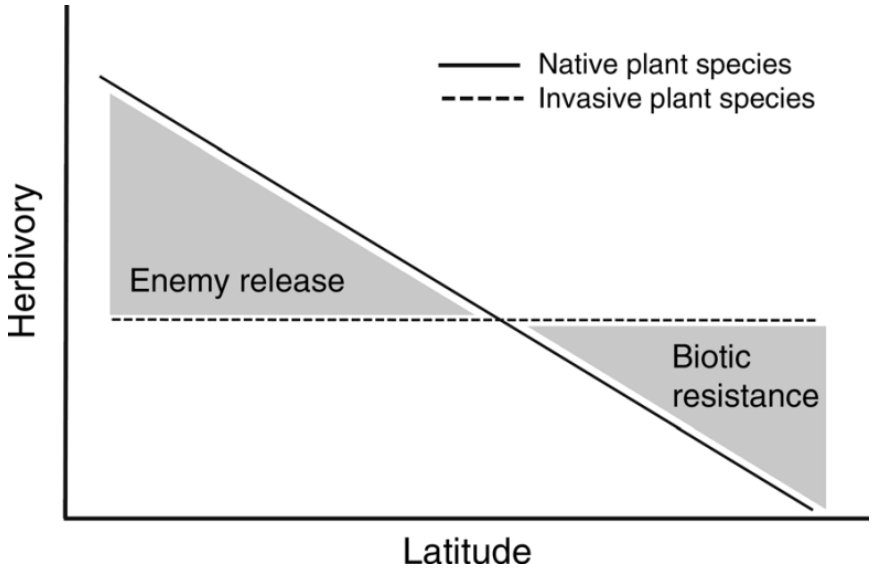

Figure 1.1. Hypothetical relationship between latitude and herbivory for native and invasive plant species. In this scenario, the invasive species experiences proportionately less herbivory than native taxa at southern latitudes (i.e., enemy release) and proportionally more at northern latitudes (i.e., biotic resistance). Adapted with permission from Cronin et al. (2015).

In my dissertation, I aimed to investigate biological invasions using a novel multitrophic and geographically broad approach to comparing biotic interactions between co-occurring native and invasive taxa. Thus, my overarching research question was: How does large-scale geographic variation in multitrophic species interactions influence invasion success? This important and unanswered research question also has an applied perspective, where I aim to provide insights that may contribute to development of novel approaches for management of invasive species around the world. Below I outline the study system used and provide a synopsis of each of my dissertation chapters.

STUDY SYSTEM

The focal organism for my dissertation was *Phragmites australis* (common reed), a large-statured macrophytic grass recently described as a model organism for studying plant invasions (Meyerson et al. 2016). *P. australis* has a global distribution and is found in a range of habitats including coastal marshes, inland lakes and rivers, wetlands, desert oases, mountains, and urban areas (Clevering and Lissner 1999; Mal and Narine 2004). A unique attribute of this species is that multiple lineages grow sympatrically in North America (Saltonstall 2002; Meyerson et al. 2009; Lambertini et al. 2012; Meyerson et al. 2012; Meyerson and Cronin 2013) ranging from native to highly invasive. The native lineage is made up of at least 14 distinct haplotypes and has been broadly distributed in North America for millennia, but is often scarce locally (Saltonstall 2002; Meadows and Saltonstall 2007; Vachon and Freeland 2011). In the past 150 years, a cryptic European lineage has spread rapidly throughout the continent, forming large monospecific populations in coastal and freshwater marshes, roadside ditches, and disturbed areas (Chambers et al. 1999; Saltonstall 2002; Howard et al. 2008). Invasion by this lineage of *P. australis* can result in severe impacts on hydrology, nutrient cycling, ecosystem function, native plant diversity, and habitat quality for fauna (Windham and Lathrop 1999; Meyerson et al. 2000; Angradi et al. 2001; Windham and Ehrenfeld 2003; Gratton and Denno 2005; Minchinton et al. 2006; Meyerson et al. 2009). As such, efforts are being made to concurrently conserve the native lineage and manage the invasive lineage; over $4.6 million per year is spent on control using conventional methods (e.g., herbicides and physical removal) (Martin and Blossey 2013), which is largely ineffective in the long-term (Hazelton et al. 2014). A third lineage known as Gulf occurs in the southern United States (Hauber et al. 2011; Lambertini et al. 2012; Meyerson et al. 2012), where it also forms rapidly-growing populations (Bhattarai and Cronin 2014). This

lineage is likely a recent arrival from Mexico, where it is native (Colin and Eguiarte 2016), although its invasive status, ecology, and impacts in North America are largely unknown.

P. australis is host to a high diversity of arthropods and microbes. For example, over 170 arthropod herbivore species have been identified in Europe, along with 26 species currently identified from North America, 21 of which are introduced (Tewksbury et al. 2002). To date, higher herbivory on the native compared to the invasive and Gulf *P. australis* lineages is a broad pattern across multiple species and guilds of *P. australis* herbivores (Lambert et al. 2007; Lambert and Casagrande 2007; Park and Blossey 2008; Cronin et al. 2015; Bhattarai et al. in review; but see Saltonstall et al. 2014). Furthermore, natural enemies of some *P. australis* herbivores are also diverse and abundant in North America (e.g., Latham and Mills 2010). Microbial communities associated with *P. australis* are rapidly being identified and a number of recent studies have described distinct oomycete, archaea, bacteria, and fungal endophyte and pathogen communities from different *P. australis* lineages in North America (Nelson and Karp 2013; Crocker et al. 2015; Yarwood et al. in press; Bowen et al. in review). Such divergent microbial communities suggest that their impacts may also differ among *P. australis* lineages, although the direction and magnitude of these effects and their importance to *P. australis* invasion success are yet to be examined (but see Crocker et al. 2015).

From a scientific perspective, the co-occurrence of conspecific lineages of *P. australis* enables robust comparisons between native, invasive, and introduced taxa by minimizing phylogenetic differences which may confound the results of other similar studies. Moreover, its global distribution and diverse community of natural enemies, competitors and mutualists makes *P. australis* ideal for examining large-scale geographic variation in multitrophic interactions.

DISSERTATION SYNOPSIS

In Chapter 2, I examined evidence for enemy release and a possible invasional meltdown over multiple trophic levels. Using a survey of 143 field sites in North America and 21 along the Atlantic coast of Europe, I examined *P. australis* patches for infestation of gall-flies in the genus *Lipara* (Diptera: Chloropidae), and *Lipara* mortality from natural enemies. Based on the frequency of damage and the direct impact on sexual reproduction (termination of flowering of infested stems), *Lipara* represent one of the most damaging and important *P. australis* herbivore groups in North America, and have been considered candidates for biological control. I hypothesized that *Lipara* infestation and mortality would differ between the introduced and native ranges and between invasive and native lineages in North America.

In Chapter 3, I used the same study system to investigate biogeographic heterogeneity in the strength of local enemy release by comparing latitudinal gradients in *Lipara* infestation between the native and invasive *P. australis* lineages. Field survey data were paired with a complementary common garden experiment to test the relative role of local adaptation and phenotypic plasticity in driving latitudinal gradients and local enemy release. I also examined the role of stem characteristics measured during *Lipara* oviposition in driving infestation. Because plants were grown in a controlled common garden environment (i.e., similar environmental conditions, flowering prevented, maternal effects minimized), latitudinal gradients in herbivory observed in the field that are also present in the common garden would be expected to have a genetic basis. In contrast, a gradient in the field that disappears in the common garden would suggest that the gradient is driven by phenotypic plasticity rather than local adaptation.

Chapter 4 represents a shift in focus from aboveground interactions to belowground interactions, with the goal of testing the net impact of soil biota on the relative performance

(biomass production and biomass allocation) of native, invasive and Gulf lineages of *P. australis*. I conducted a greenhouse experiment growing replicate populations from each of the three lineages in pots containing live or sterilized rhizosphere soil from the natal site of the *P. australis* population. Furthermore, to examine interactions among soil biota, interspecific plant competition and nutrient availability, and possible spillover effects of soil biota onto the native plant community, we grew *P. australis* at two nutrient levels and with or without native smooth cordgrass (*Spartina alterniflora*). This chapter represents the first study to evaluate plant-soil interactions of *P. australis* and their spillover onto the native community.

Finally, in Chapter 5, I summarize and synthesize the major findings of my dissertation and discuss their implications for invasion biology and management of *P. australis.* I conclude my dissertation by briefly outlining research directions I intend to pursue in the future.

CHAPTER 2
MULTITROPHIC ENEMY ESCAPE OF INVASIVE *PHRAGMITES AUSTRALIS* AND ITS INTRODUCED HERBIVORES IN NORTH AMERICA*

INTRODUCTION

A widely supported explanation for the success of invasive species is that they leave behind their coevolved natural enemies (e.g., herbivores and pathogens) when introduced to a new environment (e.g., Wolfe 2002; Mitchell and Power 2003; Liu and Stiling 2006; Castells et al. 2013), a phenomenon known as enemy-release (Elton 1958; Keane and Crawley 2002). An extension of this hypothesis, known as local enemy-release (Zheng et al. 2012), predicts that invasive species also suffer less damage from natural enemies in the introduced range compared to co-occurring, closely related native species (e.g., Dietz et al. 2004; Parker and Gilbert 2007; Cincotta et al. 2009; Funk and Throop 2009; Zheng et al. 2012; Cronin et al. 2015). This result may be driven by the inability of non-coadapted natural enemies to overcome the novel defenses of invasive species, greater palatability and nutritional quality of native species, or subtle differences in microhabitat. In contrast to the concept of enemy-release, the biotic-resistance hypothesis (Elton 1958; Parker and Hay 2005) predicts that natural enemies in the introduced range cause more mortality to invasive species than co-occurring, closely related native species (e.g., Agrawal and Kotanen 2003; Chun et al. 2010; Morrison and Hay 2011; Fan et al. 2013). This phenomenon is often attributed to the invasive species lacking effective defenses to resist

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attack by natural enemies with which they do not share an evolutionary history (Morrison and Hay 2011).

A complicating factor of both the enemy-release and biotic-resistance hypotheses is that herbivores from the region of origin of the invasive plant could also be accidentally or intentionally introduced with their invading host plant. Such a scenario does not strictly fit with either hypothesis because the introduced herbivores are presumably already coadapted with the invasive plant and are not native to the recipient community. In the novel environment, the interaction between the invasive plant and introduced herbivore species could be significantly different from in their native range. For example, herbivory of invasive plants by introduced herbivores could be greater in the introduced than native range. Although lower herbivory in the introduced than native range would not represent enemy-release *sensu stricto*, the resulting advantages to the invasive plant are likely the same. Moreover, novel indirect interactions can potentially lead to net positive effects of herbivory for the invasive host plant in the introduced range (e.g., indirect dispersal through seed predators, see Pearson et al 2000; Pearson and Ortega 2002), known as the enemy inversion hypothesis (Colautti et al. 2004).

Although tritrophic interactions have received little attention in invasion biology (Harvey et al. 2010), the strength of enemy-release or biotic-resistance may be influenced by higher trophic levels (i.e., predators and parasitoids). Differences in mortality due to natural enemies may represent an explanation for why herbivory varies between invasive and native plants, and between native and introduced ranges. Introduced herbivores may escape their own natural enemies (i.e., enemy-release), allowing them to become more prevalent on host plants in the new range (e.g., Menéndez et al. 2008; Prior and Hellmann 2013). Alternatively, if herbivores feeding on invasive plants suffer greater native natural enemy pressure than those feeding upon closely

related native hosts (e.g., Engelkes et al. 2012), this could benefit the invasive plant species through reduced herbivory (i.e., a trophic cascade).

The goal of this study was to assess the evidence supporting enemy-release and biotic-resistance at multiple trophic levels involving the common reed, *Phragmites australis* (Cav.) Trin. ex Steudel (Poales: Poaceae), monophagous gall-forming flies in the genus *Lipara* Meigen (Diptera: Chloropidae), and their natural enemies. Invasive European genotypes of *P. australis* widely overlap with the distribution of rare native genotypes in marshes and wetlands of North America (NA) (Saltonstall 2002). *Lipara* spp. are also introduced from Europe (EU) into NA. To date, there is little information on *Lipara* and their natural enemies in NA. The exceptions are the studies by Lambert et al. (2007) and Park and Blossey (2008) which found evidence suggesting *Lipara* infestation is higher on native than invasive genotypes. However, these studies were based on a comparison of three native and 16 invasive *P. australis* patches from the northeastern United States.

We surveyed 143 *P. australis* patches throughout NA and 21 patches along the Atlantic coast of EU to determine *Lipara* presence, infestation level (proportion of stems infested), performance (gall diameter and adult dry body mass), impact (stem height and flowering frequency), and mortality due to parasitoids and predators. Based on enemy-release and invasion theory, we made the following predictions: 1) infestation of *Lipara* on *P. australis* would be lower in the introduced (NA) compared to native (EU) range (i.e., enemy-release for the plant); 2) *Lipara* infestation, performance, and impact would be lower on invasive relative to native genotypes of *P. australis* in NA (i.e., local enemy-release); and 3) mortality of *Lipara* due to vertebrate and invertebrate natural enemies would be lower in NA than in EU, and on native than invasive genotypes in NA (i.e., enemy-release for the herbivore).

MATERIALS AND METHODS

Study organisms

Phragmites australis is a 2-5 m tall macrophytic grass commonly found in wetlands, rivers, salt marshes, and estuaries on every continent except Antarctica (Clevering and Lissner 1999). Although *P. australis* has been present in NA for millennia (Hansen 1978; Orson 1999), it has spread rapidly during the past 150 years. This spread has been attributed largely to the cryptic invasion of multiple invasive genotypes (Saltonstall 2002; Howard et al. 2008; Hauber et al. 2011; Lambertini et al. 2012; Meyerson and Cronin 2013; for review, see Meyerson et al. 2012), which have had profound ecological impacts, altering hydrology, ecosystem function, and degrading habitat for native species (Meyerson et al. 2000, 2009; Saltonstall 2002). The most abundant and widespread invasive genotype is known as *M* (based on an analysis of chloroplast DNA; Saltonstall 2002), which derives from EU and Asia. There are other introduced genotypes from Europe (e.g., L1 genotype; Meyerson and Cronin 2013) and we lump them all together as European invasive genotypes. Along the Gulf Coast of LA, there are also multiple non-native genotypes (Lambertini et al. 2012; Meyerson et al. 2012) and some are spreading rapidly (Bhattarai and Cronin 2014), the most common of which is known as genotype *I*. Finally, at least 14 native genotypes have been identified in NA (Saltonstall 2002; Meadows and Saltonstall 2007; Vachon and Freeland 2011), which we collectively refer to as "native genotypes" in our analyses. Because herbivory of invasive species has been shown to decrease with greater taxonomic isolation from the resident native community (Dawson et al. 2009; Hill and Kotanen 2009), our study provides a strong and conservative test of the enemy-release and biotic-resistance hypotheses by using distinct native and invasive lineages within a single species.

P. australis is host to a high diversity of arthropod herbivores in EU, where over 170 different species have been identified (Tewksbury et al. 2002). In NA, specialist native herbivores are scarce (Tewksbury et al. 2002) although generalists are common (J Cronin, G Bhattarai, W Allen and L Meyerson, unpublished data). However, the majority of herbivore damage is attributed to arthropods accidentally introduced to NA, including three species of *Lipara*: *L. pullitarsis* Doskocil and Chvala, *L. rufitarsis* Loew, and *L. similis* Schiner (Tewksbury et al. 2002; Cronin et al. 2015). The genus *Lipara* is native to EU and northern Asia and all eleven species are monophagous on *P. australis* (Grochowska 2013). *Lipara* are univoltine and a single fully-grown larva overwinters inside the senesced stem. Pupation occurs in the spring, followed shortly thereafter by adult emergence. Once mated, females oviposit on young *P. australis* shoots (Chvala et al. 1974; Reader 2003). Larvae feed internally and generally cause internodes to shorten, widen, and become engorged with nutritious parenchymatous tissue (De Bruyn 1995). Infestation of a stem is associated with strong negative effects on flowering and stem height (Lambert et al. 2007).

Four species of *Lipara* are present in EU where *P. australis* genotypes *M* and L1 are native: *L. lucens* Meigen, *L. pullitarsis*, *L. rufitarsis*, and *L. similis*. *Lipara* infestation levels (proportion of stems infested) in EU are variable; generally less than 5% of *P. australis* stems are attacked (Skuhravy 1981; Schwarzländer and Häfliger 2000; Reader 2001), although rare outbreaks of infestation levels up to 46% were reported in a survey of 19 patches over multiple years (Schwarzländer and Häfliger 2000). Moreover, *Lipara* galls in EU are frequently attacked by a high diversity of parasitoids (Nartshuk 2006), and depredated by the harvest mouse (*Micromys minutus*) and blue tit (*Cyanistes caeruleus*) (Mook 1967; Reader 2001; Nartshuk 2007).

Three, and possibly all four, of the EU *Lipara* species have been introduced into NA. *L. lucens* was identified by Sabrosky (1958) from specimens collected in Connecticut in 1931, but neither the original specimens nor any subsequent records are available. *L. similis* was likely introduced in New Jersey via packing material from Holland in 1946 (Sabrosky 1958), while the earliest records for *L. rufitarsis* and *L. pullitarsis* are from Rhode Island in 1998 and New Jersey in 2002, respectively (Tewskbury et al. 2002). To date, investigations of *Lipara* in the northeastern United States report infestation levels to be as high as 80% of stems (Balme 2000; Blossey 2003; Lambert et al. 2007; Park and Blossey 2008). *L. pullitarsis* was reported as restricted to the invasive genotype (Blossey 2003), whereas both *L. rufitarsis* and *L. similis* have been found inhabiting native and invasive genotypes, with some evidence suggesting they prefer the former (Lambert et al. 2007; Park and Blossey 2008). Furthermore, based on the frequency of damage and the direct impact on sexual reproduction, Cronin et al. (2015) suggested that *Lipara* represent one of the most damaging and important *P. australis* herbivore groups in North America. At present, there is no information on *Lipara* natural enemies in NA.

Study sites

We examined 143 *P. australis* patches throughout NA and 21 patches in Western EU (Fig. 2.1, Appendix A), for the presence of *Lipara* galls, as part of a broader herbivore survey (Cronin et al. 2015). Sampling effort in NA was concentrated along the East Coast (where *M* first appeared in herbarium records ca. 150 years ago), the Mississippi River Valley extending from Louisiana to northern Minnesota, and the Western United States. A total of 48 *M*, 1 *L1* (a recently identified invasive genotype in Canada; Meyerson and Cronin 2013), 19 *I*, and 75 native genotype patches were sampled between 2011 and 2014. Patches of different genotypes often occurred in the same watershed but were rarely intermixed. In EU, patches (all genotype *M*)

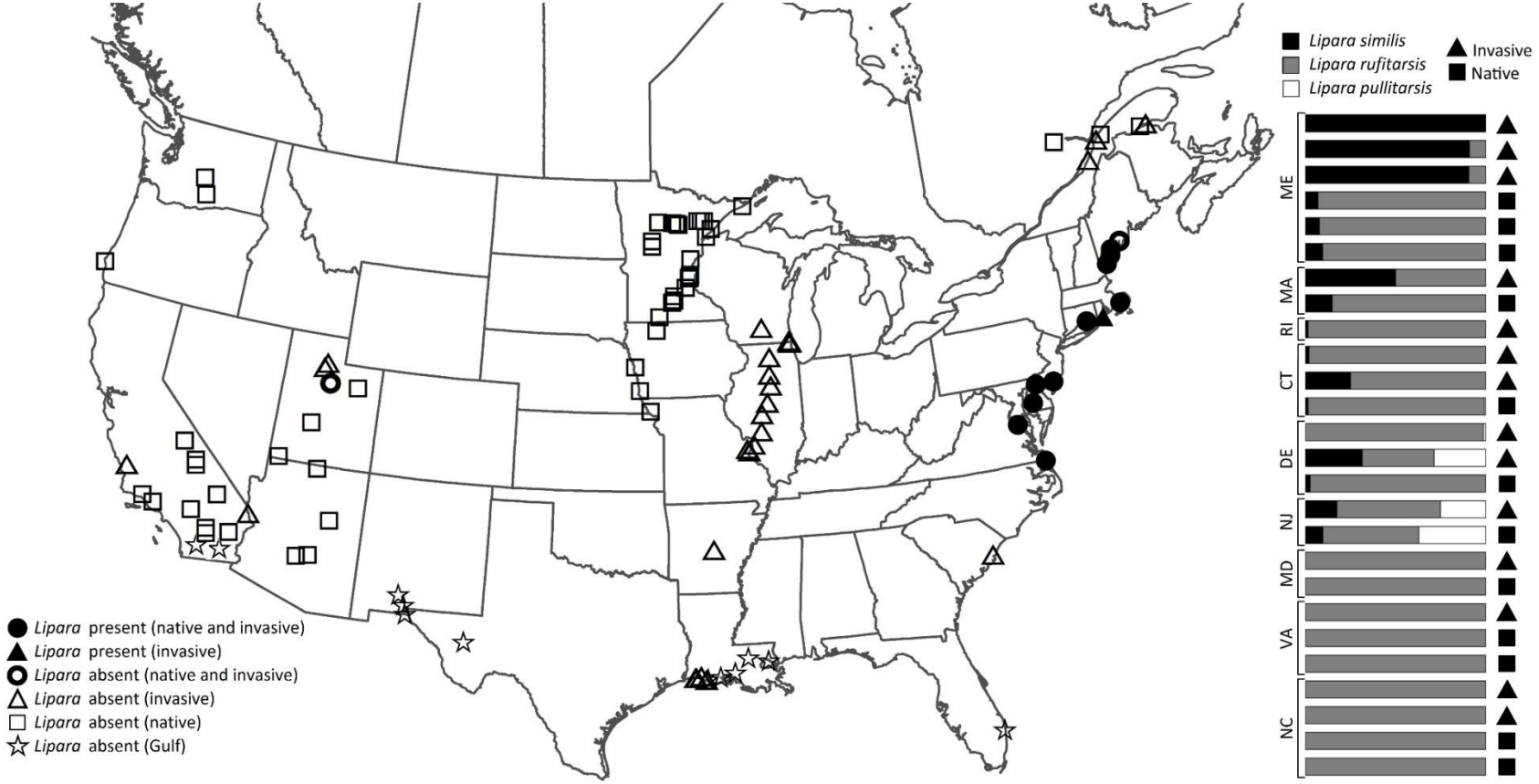

Figure 2.1. *Phragmites australis* sampling sites and the distribution of *Lipara* species in North America. The relative abundance (proportion of collected stems inhabited by each *Lipara* species) of *Lipara* species is shown for patches occupied by *Lipara*.

were selected to complement the geographic range of those in NA. Leaf material from each patch was collected for later determination of genotype (based on chloroplast DNA) using the methods of Saltonstall (2002) but with modifications outlined in Kulmatiski et al. (2010).

Data collection

Lipara distribution and infestation level

All *P. australis* patches were inspected by a team of 2-4 investigators for the presence of *Lipara* galls. The minimum inspection period was 5-10 minutes, but if *Lipara* appeared absent or scarce, 30-60 minutes was spent searching the patch to confirm presence or absence, and to maximize gall collection for the study. Sampling in NA was conducted during four different seasons: summer 2012 (July 31 – August 20), winter 2013 (March 1 – April 20), summer 2013 (August 1 – 24), and summer 2014 (August 17 – 26). Most patches were only sampled once, but some were sampled on a second occasion to collect overwintering galls (Appendix A). EU patches were visited in summer 2012 (July 22 – August 26). We note here that all gall collections were made during the same *Lipara* generation (summer 2012 and winter 2013), minimizing any temporal variability in the data.

The proportion of stems infested with *Lipara* per *P. australis* patch was estimated for all patches in NA and EU where *Lipara* were found (Fig. 2.1). Within each patch, we walked three separate transects from the edge to interior, examining the three closest stems every 2 m for the presence of a *Lipara* gall, for a total of 150 stems (50 stems per transect). Patch size (estimated by walking the patch exterior with a handheld GPS or using aerial images for very large patches) and stem density (four replicates of stems per 0.25 m^2 quadrat) were also recorded at sites visited in summer 2012. Initial analyses showed that patch size and stem density were unrelated to

Lipara infestation (Appendix A), so these data were no longer collected in subsequent (winter) surveys or included in later analyses.

Lipara species identity, natural enemies, and performance

To examine *Lipara* species composition, parasitism and predation, and performance in native versus invasive *P. australis* patches in NA, galled stems were collected from *Lipara*-infested patches (Fig. 2.1). In the summer of 2012, 70.1 ± 8.2 galls (mean ± S.E.; range: 13 to 119; number depended on availability) were collected from each of 17 patches (9 native, 8 invasive; Appendix A). All stems were dissected and *Lipara* larvae were identified to species (see Chvala et al. 1974) and examined for parasitism. A second collection of galls (174.0 ± 11.2 per patch; range: 65 to 275) was made during late winter of 2013 from 21 patches (11 native, 10 invasive) in order to rear gall inhabitants. As noted previously, galls from this latter collection (winter) represented the same generation of *Lipara* as the previous (summer) collection. These winter galls were placed in individual Ziploc bags in an environmental chamber (25 °C, 95% RH, 16:8 hour light:dark). Bags were checked weekly and scored based on whether a *Lipara* adult (identified to species), parasitoid, or predator emerged. Galls exhibiting pecking or chewing damage, and from which no *Lipara* emerged, were considered to have been successfully depredated by unidentified mammalian or avian predators. If no *Lipara* emerged, galls were dissected to confirm mortality.

From galls collected in the winter of 2013, *L. rufitarsis* was the only species reared in sufficient numbers to test differences in performance between native and invasive *P. australis* genotypes. We used dry body mass of emerged adults as a proxy for herbivore performance (see Taylor et al. 1998; Tammaru et al. 2002). For each patch with sufficient numbers, 10 male and 10 female *L. rufitarsis* adults were dried in an oven (60 °C for 48 hours) and weighed to the

nearest 0.1 mg using a Mettler microbalance. Ten individuals of each sex were used because single flies were too light to register an accurate measurement on the scale. Mean gall diameter (another measure of larval performance, see Stille 1984; McKinnon et al. 1999; Sopow and Quiring 2001) for each patch visited in the winter of 2013 was estimated from the average maximum diameter of 10 *L. rufitarsis* galls per patch (measured to the nearest 0.1 mm).

Stem height and flowering

For the most common gall species, *L. rufitarsis*, we assessed whether galled and non-galled stems differed in stem height and flowering frequency, and how this varied with *P. australis* genotype. At each NA patch visited during the winter of 2013 (11 native, 9 invasive, spanning the known range of *Lipara* in NA), the heights of 10 galled and 10 non-galled stems, randomly selected along the sampling transects, were measured to the nearest cm. In addition, flowering of non-galled stems was quantified at all sites where *Lipara* were present by examining 150 random stems along the sampling transects. All galled stems encountered (13 galls minimum, see Appendix A) were also scored for presence or absence of flowers.

Data analysis

We tested whether the *Lipara* infestation level (proportion of stems infested) per patch differed among the three phylogeographic groups, NA native (n = 12), NA invasive (n = 14), and EU native (n = 5). We only used sites where *Lipara* was present and the data were analysed using a one-way ANOVA in R version 3.0.3 (R Development Core Team 2015). The distribution of the proportions of stems infested with *Lipara* galls per patch was normalized using the logit transformation and pairwise differences among phylogeographic groups were assessed with a Tukey's test. To assess whether a particular *Lipara* species was driving differences in infestation levels we compared *Lipara* species composition between native and invasive *P. australis*

genotypes in NA (composition data were unavailable for EU). To do this we calculated the infestation level of each individual *Lipara* species as the product of each species' proportional abundance (based on emergences from collected galls) and the proportion of stems infested by all *Lipara* species combined (from the field census). Infestation levels were compared between native (n = 12) and invasive (n = 14) patches for each *Lipara* species using a MANOVA with *P. australis* genotype as the categorical variable. The distribution of infestation levels was normalized using the logit transformation.

Predation by vertebrates was compared between *Lipara*-infested native (n = 11) and invasive (n = 10) *P. australis* patches in NA using a generalized linear model. Whether or not a gall was depredated was the dependent variable with a quasibinomial link function to account for overdispersion (McCullagh and Nelder 1989). *P. australis* genotype (native, invasive) was a fixed factor, and mean gall diameter and patch size (see below) were included as covariates in the model. The model was analyzed using R, which provided *t*-statistics as output. Gall size and patch size are known to influence natural enemies (e.g., Weis and Abrahamson 1986; Morrison et al. 2010, respectively) but have never before been tested with *Lipara*. We tested for a difference in predation success (the proportion of attacks resulting in the disappearance or death of *Lipara*) between native and invasive *P. australis* genotypes using a *t*-test.

To assess whether adult *L. rufitarsis* body mass differed between *P. australis* genotypes (11 native, 9 invasive patches), we used a two-way ANCOVA in R. Genotype and *L. rufitarsis* sex were fixed factors in the model; the latter was included to account for possible sexual dimorphism within the species. Gall diameter was added as a covariate. Mean diameter of *L. rufitarsis* galls on native and invasive genotypes was also compared using a *t*-test as an additional performance measure.

To examine the potential impact of *L. rufitarsis* on *P. australis*, we tested if the mean height of galled stems was shorter than non-galled stems for both native and invasive genotypes (11 and 9 patches respectively) using *t*-tests. The proportional reduction in stem height (= [galled - non-galled]/non-galled) was also compared between genotypes using a *t*-test to examine if the mean reduction in stem height was greater for native or invasive *P. australis*. Finally, we calculated the impact of *Lipara* on sexual reproduction at each site as the product of flowering frequency of non-galled stems and the proportion of stems infested by *Lipara* (from the field survey). Because galled stems always failed to flower, this metric represents the proportional reduction in flowering due to the occurrence of galls. We compared *Lipara* impact on sexual reproduction between native (n = 12) and invasive (n = 14) genotypes using a *t*-test.

RESULTS

Lipara distribution and infestation level

Lipara were found only on the east coast of NA between latitudes of 36.5° and 43.8°, ranging from northern North Carolina to central Maine (Fig. 2.1). Galls were absent from all other locations. All three *Lipara* species were found to infest native and invasive *P. australis* genotypes. *L. rufitarsis* was the most widespread species, and the only species found south of New Jersey. *L. similis* increased in abundance in northern invasive patches and was the most dominant *Lipara* species in Massachusetts and Maine. *L. pullitarsis* was present in only five patches from New Jersey to Connecticut. In Europe, *Lipara* were present in all countries surveyed (Appendix A), ranging from Portugal (40.6°) to Norway (59.3°), but their overall distribution was patchy (present in only 5 of 21 patches surveyed).

Within the occupied range, the overall proportion of *P. australis* stems infested with *Lipara* differed significantly among NA native, NA invasive, and EU native patches ($F_{2, 28}$ =

25.73, $P < 0.001$, Fig. 2.2 and 2.3a). In native *P. australis* patches, 32.0 ± 3.9% (mean ± S.E.) of stems had a *Lipara* gall, which was three and 40 times higher than the infestation levels for NA invasive (10.6 ± 2.8%) and EU native (0.8 ± 0.1%) patches, respectively (Fig. 2.2 and 2.3a, all comparisons $P < 0.001$). For the European genotypes, the proportion of stems with galls was over thirteen times higher in the invaded range compared to the native range ($P = 0.002$).

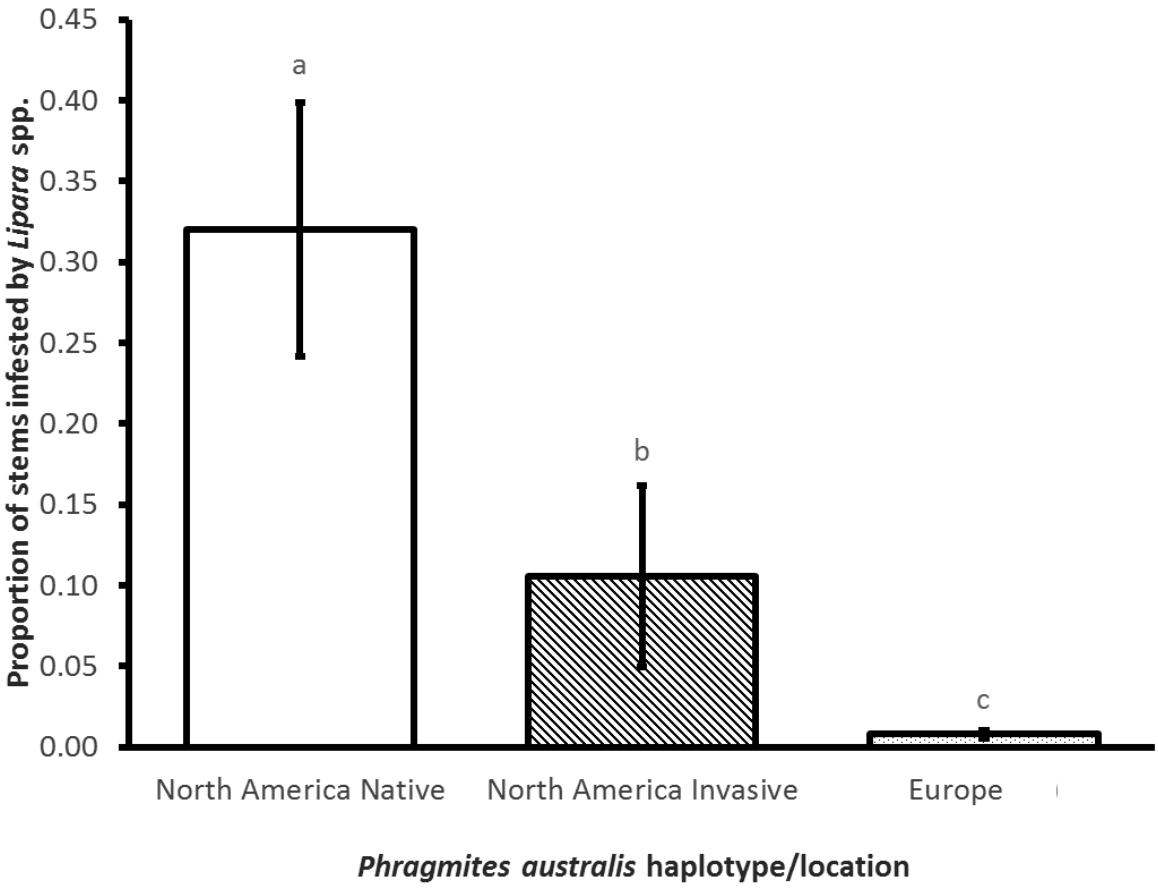

Figure 2.2. Mean proportion of stems infested by *Lipara* (± 95% CI) in North American native, North American invasive, and European *Phragmites australis* patches. Different letters indicate significant differences between genotype means ($P < 0.05$).

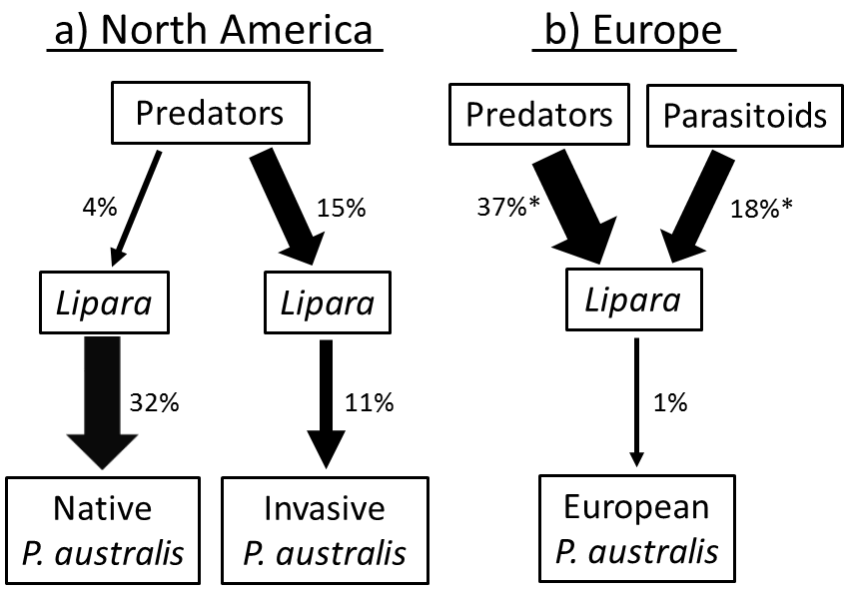

Figure 2.3. Schematic diagram illustrating biotic interaction strengths between *Phragmites australis*, *Lipara*, and predators/parasitoids of *Lipara* in North America and Europe, at sites where *Lipara* were present. Parasitoids were absent in North America. Arrow thickness represents the strength of each interaction, which is also shown by the percentage beside each line (i.e., % of *Lipara* galls depredated or parasitized; % of *P. australis* stems infested by *Lipara*). *Predation and parasitism of *Lipara* in Europe is based on an overall average of 25 data points collated from Abraham and Carstensen 1982; Athen and Tsharntke 1991; Tscharntke 1994; Schwarzlander and Hafliger 2000; Reader 2001; Reader 2003 (Appendix B).

Lipara species composition differed significantly between native and invasive genotypes in NA when analysed using MANOVA (Wilks's Lambda $F_{3,22} = 3.87$, $P = 0.023$, Fig. 2.4). This difference in species composition was brought about by *L. rufitarsis*, which was over five times more abundant in native than invasive *P. australis* patches ($F_{1,24} = 12.04$, $P = 0.002$; Fig. 2.4). 92 ± 7.7% of galls collected from native *P. australis* were identified as containing *L. rufitarsis*, compared to only 67 ± 20.8% of the invasive *P. australis* galls. Infestation levels of *L. similis* ($F_{1,24} = 0.08$, $P = 0.782$) and *L. pullitarsis* ($F_{1,24} = 0.01$, $P = 0.946$) did not differ significantly between native and invasive *P. australis* genotypes (Fig. 2.4).

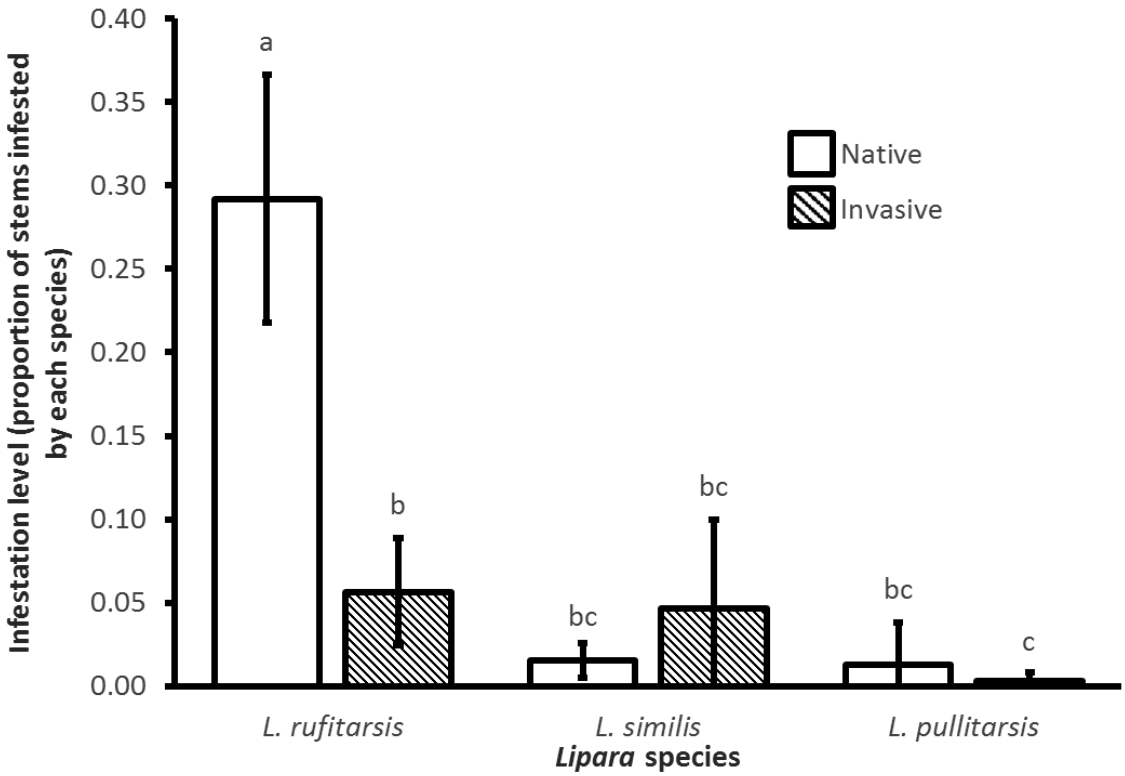

Figure 2.4. Mean proportion of collected stems inhabited by each *Lipara* species (± 95% CI) in North American native and invasive *Phragmites australis* patches. Different letters indicate significant differences between means ($P < 0.05$).

Lipara parasitism and predation

Of the 1,663 NA galls inspected, we found no evidence of mortality from arthropod parasitoids or predators. In contrast, vertebrate predators successfully attacked 14.8 ± 6.2% of *Lipara* galls on the invasive genotype and 3.5 ± 2.6% of galls on native genotypes, however this fourfold difference was non-significant ($t = -0.75$, $P = 0.464$, Fig. 2.3a and 2.5a). Gall diameter ($t = -0.68$, $P = 0.684$) and patch size ($t = 0.21$, $P = 0.837$) were not related to the successful predation level. Not all attacked galls (as evidenced by pecking or chewing damage) resulted in the death of the *Lipara* inhabitant. Seventy ± 22.7% of attacks on galls of invasive genotype and 66 ± 32.3% of attacks on native genotypes resulted in the disappearance or death of *Lipara*; a difference that was non-significant ($t_{13} = -0.21$, $P = 0.840$).

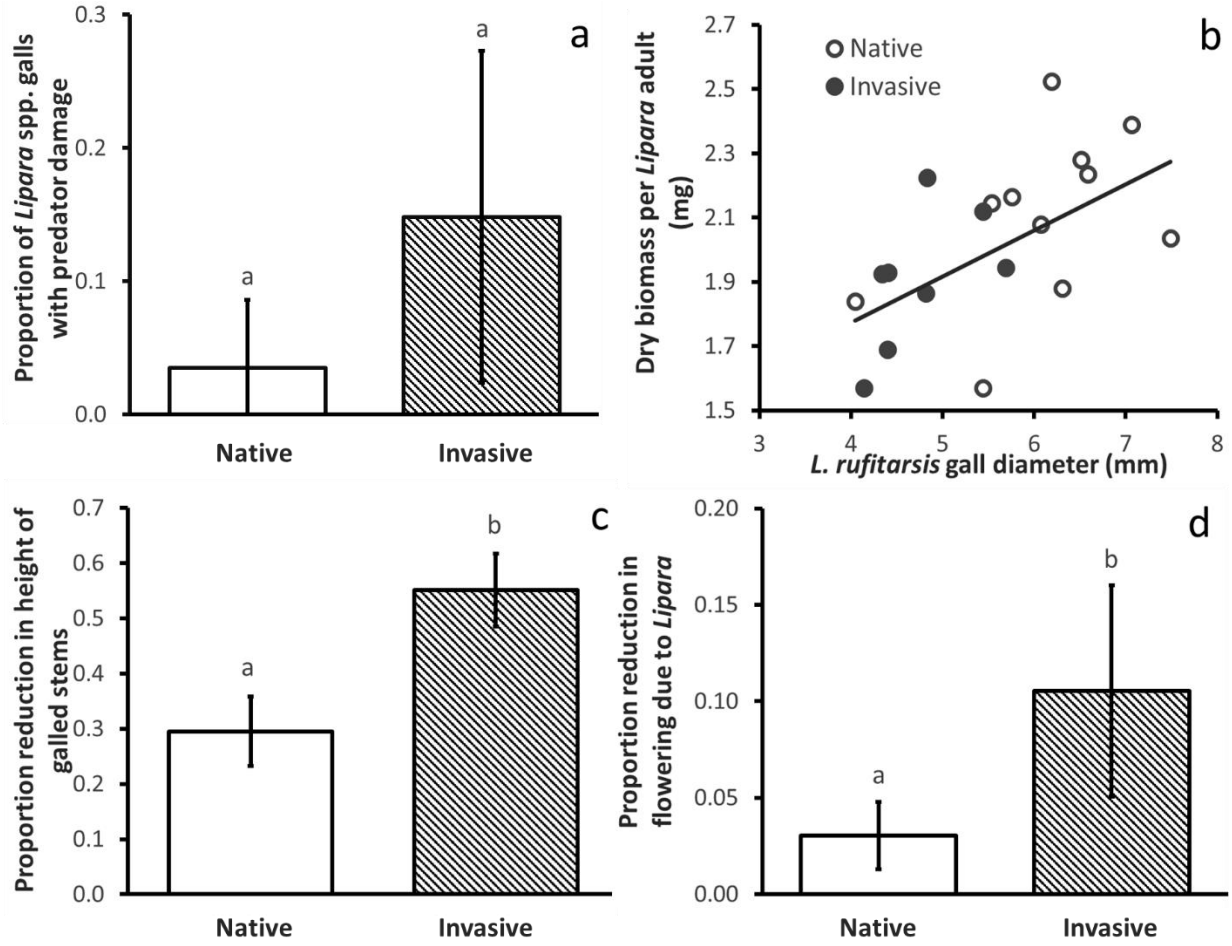

Figure 2.5. For native and invasive *Phragmites australis* genotypes in North America, the (a) proportion of *Lipara* galls attacked by mammal or bird predators; (b) relationship between gall diameter and dry body mass of *L. rufitarsis*; (c) proportional reduction in height of stems infested by *L. rufitarsis*; and (d) proportional reduction in flowering frequency due to *L. rufitarsis*. Reported are the means ± 95% CI per patch. Different letters indicate significant differences between genotype means ($P < 0.05$).

Lipara performance

Dry body mass of *L. rufitarsis* adults was 13% higher for individuals reared from native than invasive genotypes, but this result was non-significant ($F_{1,35} = 0.95$, $P = 0.337$). Female *Lipara* (2.6 ± 0.2 mg) weighed almost twice as much as males (1.4 ± 0.1 mg) ($F_{1,35} = 197.34$, $P <$ 0.001). A marginally significant positive correlation between the covariate gall diameter and

body mass was detected ($F_{1,35}$ = 3.48, P = 0.071, Fig 2.5b). If we removed gall diameter as a covariate in the model, genotype also became significant ($F_{1,36}$ = 7.00, P = 0.012) suggesting that differences in *Lipara* performance between genotypes is due to the effects of genotype on gall size. *L. rufitarsis* galls were 34% larger on the native than invasive genotypes (t_{18} = 5.75, P < 0.001, Fig. 2.5b).

***P. australis* stem heights and flowering**

Stems of the invasive *P. australis* genotypes with a *L. rufitarsis* gall were 55 ± 6.6% shorter than non-galled stems (t_{10} = 7.82, P < 0.001). In comparison, native stems with galls were 30 ± 6.3% shorter than non-galled stems (t_8 = 10.59, P < 0.001). The degree of reduction in stem height when a gall was present was significantly greater for the invasive than native genotype (t_{16} = 5.53, P < 0.001, Fig. 2.5c). No galled stems were observed to have flowered. Invasive *P. australis* genotypes suffered a 10.5 ± 2.7% reduction in flowering due to *Lipara*, almost 3.5 times greater than the 3.0 ± 0.9% reduction suffered by native genotypes (t_{24} = -2.43, P = 0.023, Fig 2.5d). However, flowering of non-galled stems was over twofold higher in patches of invasive than native genotypes (t_{24} = -3.03, P = 0.006).

DISCUSSION

Despite a recent increase in the number of studies involving multi-species introductions into the same community (e.g., Rand and Louda 2004; Lau and Strauss 2005; Dangremond et al. 2010; Green et al. 2011; Stricker and Stiling 2012), our understanding is still limited as to how species interactions change between the native and introduced ranges and the potential implications for invaded native communities. With invasive species expected to become more prevalent (Levine and D'Antonio 2003), it is also likely that trophic interactions involving multiple introduced species will become commonplace. The tritrophic interactions between *P.*

australis, *Lipara* spp. and their natural enemies in EU and NA are summarized in Fig. 2.3. Support for our first prediction varied regionally; *P. australis* was released from *Lipara* throughout most of NA (Fig. 2.1), but our study also showed that along the Atlantic coast the attack of invasive *P. australis* by introduced *Lipara* species was higher in the novel than ancestral range. Escape from their predators and parasitoids in the introduced range likely allowed *Lipara* to achieve higher infestation levels (proportion of stems infested) in NA than EU, supporting our third prediction of enemy-release for the gall-forming herbivores. In the novel range, we found that invasive *P. australis* suffered lower attack from the introduced *Lipara* than closely related native *P. australis*, supporting the local enemy-release hypothesis and our second prediction. Such a result is likely due to a lack of coevolutionary history between native *P. australis* and introduced *Lipara*, but native predators that cause higher mortality of *Lipara* on invasive plants could also contribute to the difference in herbivory between native and invasive plants in the novel range. Our study points to the complex interactions that arise when two or more interacting species are introduced into a novel environment, and that a multitrophic framework is required when investigating the influence of biotic interactions on invasion success.

The enemy inversion hypothesis posits that the effects of introduced herbivores on invasive plants may be reduced or even reversed due to novel interactions in their new environment (Pearson et al. 2000; Pearson and Ortega 2002; Colautti et al. 2004). Our study did not support this prediction. *Lipara* herbivory on European genotypes of *P. australis* was over thirteen times higher in their introduced range (NA) in comparison to their native range (EU). We postulate that the higher infestation level in NA found in our study may be driven by classical enemy-release of *Lipara* from their EU arthropod predators and parasitoids. The total

absence of parasitism in our sampled galls provides stark contrast to parasitism rates in EU previously reported to be 15-26% for *L. rufitarsis* (Tscharntke 1994; Reader 2001; Reader 2003), 22% for *L. similis* (Tscharntke 1994; Schwarzländer and Häfliger 2000), 0-59% for *L. pullitarsis* (Abraham and Carstensen 1982; Tscharntke 1994; Athen and Tsharntke 1999), and averaging 18% across all *Lipara* species and studies (Fig. 2.3b, Appendix A). Moreover, Nartshuk (2006) reported 33 parasitoid species attacking galls of these *Lipara* species in EU. We found no evidence that any of these natural enemies of *Lipara* have been introduced to NA, nor does it seem that native parasitoids have incorporated these novel prey into their host range. Furthermore, predation on *Lipara* galls by unidentified mammalian or avian predators on the invasive and native *P. australis* genotypes in NA was over two and nine times lower, respectively, than found for *Lipara* galls in EU where the attack rate averaged 37% across three years (Reader 2001).

Based on our study, the distribution of *Lipara* in NA is restricted to the Atlantic coast from North Carolina to Maine. This finding expands the known range of *Lipara*, previously reported as far south as New Jersey (Tewksbury et al. 2002). Moreover, unpublished reports by experts on *P. australis* (C. Rohal and E. Hazelton, pers. comm.) suggest that *Lipara* (species as yet unidentified) are present in Utah. Given the vast area that *P. australis* covers in NA, it is no surprise that *Lipara* has recently begun appearing in isolated areas further west of its site of arrival on this continent, potentially facilitated by the spread of the invasive genotype. Interestingly, contrary to the report by Blossey (2003), we did find *L. pullitarsis* infesting stems of native *P. australis* genotypes. No *Lipara* were found at any of the surveyed patches north of Yarmouth, Maine (43.8°) (Fig. 2.1; see also Lambert et al. 2007). However, *Lipara* (species unidentified) were present in Norway during our European survey at a latitude of 59.3° and are

common at high latitudes (Chvala et al. 1974). This distribution suggests *Lipara* may be able to tolerate colder conditions and that their current northern distribution in NA might not be limited by physiological tolerances. In contrast, physiological tolerances may be limiting the southern extent of *Lipara* in NA. A single *L. similis* observation in Israel (approximately 31.7°) (Nartshuk 1984) is the only location worldwide in which *Lipara* has been reported further south than our North Carolina patches (36.5°), suggesting that *Lipara* may not be tolerant of hotter climates, such as the Gulf Coast region or southwestern United States.

Lipara appear to have considerable impact on *P. australis* sexual reproduction and growth in NA; flowering of infested stems was zero regardless of genotype, suggesting a strong negative effect of *Lipara* on sexual reproduction of infested stems, which is important to the spread of *P. australis* (Brisson et al. 2008; McCormick et al. 2010). *Lipara* reduced flowering by 10.5% for the invasive genotype and 3.0% for native genotypes, a difference of over threefold. Furthermore, heights of *L. rufitarsis*-infested stems of native and invasive genotypes were also 30% and 55% shorter than non-galled stems, respectively (see also Lambert et al. 2007). At present, it is unknown whether *L. rufitarsis* caused reductions in stem height, or if ovipositing females simply selected stems predisposed to achieving shorter heights. Some support is provided for the latter, as *L. rufitarsis* prefer stems with a small basal diameter (De Bruyn 1993; De Bruyn 1994; Tscharntke 1994), a trait strongly correlated with stem height (Reader 2001). Long-term effects of *Lipara* and other herbivores on the population-level response of native and invasive *P. australis* genotypes are currently unknown and should be a focus of future research efforts, particularly for potential biological control agents.

We found support for local enemy-release of invasive *P. australis* in the introduced range relative to co-occurring native genotypes. Native *P. australis* genotypes suffered threefold

greater herbivory from *Lipara* than co-occurring invasive genotypes in NA, which was attributed to a fivefold greater infestation level of *L. rufitarsis* in native compared to invasive patches. The pattern of higher herbivory of native genotypes was consistent with previous studies of three native *P. australis* patches from the northeastern United States (Lambert et al. 2007; Park and Blossey 2008) and is consistent with findings involving other invasive species (e.g., Dietz et al. 2004; Parker and Gilbert 2007; Cincotta et al. 2009; Funk and Throop 2009; Zheng et al. 2012). Cronin et al. (2015) also found that native *P. australis* genotypes in NA suffered greater herbivory from the entire guild of internal feeding herbivores than the invasive genotype, and the same pattern was observed for the widespread and abundant non-native aphid, *Hyalopterus pruni,* and all chewing herbivores combined. These results suggest that native *P. australis*-inhabited marshes are susceptible to invasion by *Lipara* and possibly other introduced herbivores. Although invasive *P. australis* suffers lower herbivory compared to native genotypes, this does not fit within the strict definition of enemy-release or biotic-resistance, because *Lipara* are also introduced to NA. However, the ecological implications of such a pattern on invasion success are the same – an advantage to the invasive plant in its novel range. We suggest that the conceptual framework of enemy-release and biotic-resistance be expanded to also include the effects of introduced herbivores, and that further studies are needed examining novel communities inhabited by multiple interacting native and introduced species.

We offer several possible explanations for why *Lipara*, and possibly other introduced herbivores of *P. australis*, are responsible for greater levels of herbivory on native than invasive genotypes in NA. First, the difference in infestation levels could be explained by the influence of higher trophic levels (i.e., natural enemies; see Fig. 2.3). We found higher levels of predation by vertebrates on galls of the invasive genotype (14.8%) relative to galls of native genotypes

(3.5%). While this difference was not statistically significant, the large effect size suggests *Lipara* feeding on native genotypes may be released from top-down pressure, whereas invasive *P. australis* may benefit from greater top-down control of herbivores (i.e., a trophic cascade; see Fig. 2.3). To our knowledge, this study is the first to show that higher trophic levels may impact invasion success in this manner. Second, the invasive genotype has coevolved with *Lipara* in EU and Asia and may therefore have evolved resistance to attack by *Lipara.* In contrast, *Lipara* have only recently been introduced to NA and native genotypes have had little time to adapt defenses to these novel herbivores. For example, the putative defense trait of leaf toughness is greater in invasive than native *P. australis* genotypes (Cronin et al. 2015). Such coevolved plant–herbivore interactions provide bottom-up control of native herbivores, but may allow for outbreaks of newly-associated introduced herbivores (Gandhi and Herms 2009; Desurmont et al. 2011). Thus, a lack of a coevolutionary history with *Lipara* could result in a lack of specialized defenses with which native *P. australis* genotypes can resist infestation. Furthermore, differences in palatability or nutritional quality may contribute to the difference in herbivory between native and invasive *P. australis* genotypes. Gall diameter and body mass, often indicators of host nutritional quality (e.g., Stille 1984; Taylor et al. 1998; McKinnon et al. 1999; Sopow and Quiring 2001; Tammaru et al. 2002), were 34% and 13% higher, respectively, on native than invasive genotypes. Third, previous studies have shown that *L. rufitarsis* is more commonly found infesting *P. australis* shoots with a thin basal diameter (De Bruyn 1993; De Bruyn 1994; Tscharntke 1994). The typically thinner stems of the native genotypes (Lambert et al. 2007) may predispose them to attack by *L. rufitarsis*.

The pattern of greater herbivory on native than invasive genotypes of *P. australis* in NA is emerging as a common phenomenon across multiple species and guilds of introduced

herbivores (this paper; Lambert et al. 2007; Lambert and Casagrande 2007; Park and Blossey 2008; Cronin et al. 2015, but see Saltonstall et al. 2014). This trend suggests that *Lipara* and perhaps other herbivore species may be involved in an invasional meltdown (Simberloff and Von Holle 1999), the process whereby multiple invasive species facilitate one another's spread or exacerbate their impact on native species. Invasive plant species have been shown to facilitate the growth and spread of introduced herbivore populations, leading to negative effects on closely related native plant species via apparent competition (Colautti et al. 2004; Rand and Louda 2004; Lau and Strauss 2005; Dangremond et al. 2010; Lambert and Dudley 2014). Likewise, introduced generalist herbivores can also indirectly facilitate the growth and spread of invasive plant species by preferentially feeding on their native competitors (Parker et al. 2006; Relva et al. 2010). An alternative outcome is invasional antagonism, where invasive species inhibit one another through competition (Belote and Weltzin 2006) or herbivory (La Pierre et al. 2010; Stricker and Stiling 2012). In the situation of *P. australis*, despite the impact of *Lipara* on sexual reproduction, it is unlikely that *Lipara* are limiting the spread of invasive *P. australis* due to the plant's rapid clonal growth, high stem density, and greater biomass and flowering frequency relative to native genotypes (see Mozdzer et al. 2013 for review). However, the sheer pervasiveness of the invasive genotypes combined with the escape from parasitoids that *Lipara* has experienced in NA has possibly facilitated the spread of these herbivores to the relatively rare native *P. australis* genotypes. A key research need is to determine effects of herbivory on competitive outcomes among invasive and native genotypes at the population level, and if the higher relative level of herbivory experienced by native genotypes is contributing to their decline and disappearance in eastern NA.

CHAPTER 3
BIOGEOGRAPHY OF A PLANT INVASION: DRIVERS OF LATITUDINAL VARIATION IN LOCAL ENEMY RELEASE

INTRODUCTION

One of the most general and recognizable patterns in ecology is the latitudinal diversity gradient (Pianka 1966; Hillebrand 2004). Ecologists have hypothesized that this phenomenon should contribute to the evolution of stronger species interactions (e.g., herbivory, competition, predation, mutualisms) at lower than higher latitudes (Dobzhansky 1950; Coley and Aide 1991; Schemske et al. 2009). A meta-analysis by Moles et al. (2011) found a significant negative latitudinal gradient in herbivore damage for only 37% of studies, while an additional 21% reported a significant positive latitudinal gradient. Clearly, latitudinal gradients in herbivory are not always observed, and the direction of those gradients which do exist is variable.

Species interactions are likely important in the establishment and spread of invasive species, as predicted by the contrasting local enemy-release (invasive species suffer less damage from natural enemies in their introduced range relative to co-occurring native species; Zheng et al. 2012) and biotic resistance hypotheses (natural enemies in the introduced range cause greater damage to invasive species than co-occurring native species; Elton 1958; Levine et al. 2004). Thus, if sympatric native and invasive plant species exhibit dissimilar or non-parallel relationships between herbivory and latitude, this could lead to heterogeneity in the strength of local enemy release and biotic resistance at a biogeographic scale (Bezemer et al. 2014; Cronin et al. 2015). For example, Cronin et al. (2015) examined latitudinal gradients in herbivory from several herbivore guilds on co-occurring native and invasive lineages of the wetland grass *Phragmites australis* (Cav.) Trin. ex Steudel (Poaceae) in North America. Chewing damage and incidence of internal feeding herbivores (all species combined) was lower on average for the

invasive than native lineage. However, damage decreased with increasing latitude for the native lineage, but was independent of latitude for the invasive lineage. Consequently, local enemy release was strongest for the invasive lineage at southern latitudes (i.e., lowest biotic resistance).

A combination of field surveys and common garden studies is a powerful approach to determining whether environmental gradients in herbivory are evolved (i.e., owing to natural selection and local adaptation) and/or phenotypically plastic responses to the local environment (e.g., Woods et al. 2012; Hiura and Nakamura 2013; Bhattarai et al. in review). A gradient in the field that disappears in the common garden would suggest that phenotypic plasticity is the underlying cause for the gradient. Alternatively, the absence of a gradient in the field but the presence of one in the common garden would suggest that environmental variability obscures evidence of local adaptation.

The goal of this study was to compare the strength and direction of latitudinal gradients in herbivory between native and invasive plants and to investigate whether gradients are driven by local adaptation and/or phenotypic plasticity. We focused on the native and invasive lineages of *P. australis* and a specialist gall-forming fly *Lipara rufitarsis* Loew (Diptera: Chloropidae). We surveyed 25 *P. australis* populations (12 native, 13 invasive) along the east coast of North America from North Carolina (36.5°) to Maine (43.6°) to examine biogeographic variation in the proportion of stems with galls of *L. rufitarsis*. We also ran a complementary common garden experiment examining *L. rufitarsis* herbivory of 74 *P. australis* populations sourced from throughout North America. Specifically, we tested the following predictions: 1) native and invasive *P. australis* lineages will exhibit non-parallel latitudinal gradients in the proportion of stems with galls (i.e., biogeographic heterogeneity in relative strength of herbivory); 2) the proportion of stems with galls will be lower on the invasive than native lineage in the field (i.e.,

the local enemy release hypothesis); 3) the same patterns will be reflected in a complementary common garden experiment (i.e., gradients in herbivory have a genetic basis); and 4) the proximal mechanism underlying variation in the proportion of stems with galls is related to stem characteristics at the time of *L. rufitarsis* oviposition.

MATERIALS AND METHODS

Study organisms

Phragmites australis is a model organism for studying plant invasions (Meyerson et al. 2016). It is a large-statured perennial grass which forms dense stands in the littoral zone of lakes, rivers, and fresh- and saltwater marshes, and is virtually cosmopolitan in its distribution (Lambertini et al. 2006). A native *P. australis* lineage has been present in North America for millennia and consists of at least 14 different haplotypes (Saltonstall 2002; Meadows and Saltonstall 2007; Vachon and Freeland 2011). However, over the last 150 years, an invasive lineage of *P. australis* from Europe has spread throughout North America (Chambers et al. 1999; Saltonstall 2002; Howard et al. 2008; Meyerson et al. 2009; Meyerson et al. 2012). An additional lineage (known as Gulf) is located in the Gulf Coast region (Hauber et al. 2011; Lambertini et al. 2012; Meyerson et al. 2012), where it also forms rapidly-growing monotypic populations (Bhattarai and Cronin 2014). However, its status as an invader is unclear. The co-occurrence of conspecific lineages enables robust comparison between native and invasive taxa by minimizing phylogenetic differences between taxa.

Herbivory of *P. australis* in North America is mostly attributed to accidentally introduced arthropods, including three species of monophagous and univoltine *Lipara* gall-flies introduced from Europe: *L. pullitarsis* Doskocil and Chvala, *L. rufitarsis*, and *L. similis* Schiner (Tewksbury et al. 2002; Allen et al. 2015). *Lipara* adults emerge in the spring and mated females oviposit on

young *P. australis* shoots and the resultant larvae induce distinctive cigar-shaped galls in the apical part of stems (Chvala et al. 1974). A single fully-grown larva overwinters inside the senesced stem, before pupation occurs in the spring. All three *Lipara* species in North America attack the native and invasive lineages of *P. australis* (Allen et al. 2015), but higher herbivory has consistently been reported on the former, with up to 80% of stems with galls per population (Balme 2000; Lambert et al. 2007; Park and Blossey 2008; Allen et al. 2015). *L. rufitarsis* is the most widespread and abundant species, occurring from North Carolina to Maine and inhabiting 79% of galls (Allen et al. 2015). Stems infested by *Lipara* have reduced size and never produce a panicle (Lambert et al. 2007; Park and Blossey 2008; Blossey 2014; Allen et al. 2015). Based on the frequency of damage and the direct impact on sexual reproduction, *Lipara* is one of the most damaging and important herbivores of *P. australis* in North America (Cronin et al. 2015).

Field survey

To examine latitudinal variation in the proportion of stems with *L. rufitarsis* galls, we surveyed 25 *P. australis* populations (12 native, 13 invasive) along the East Coast of the United States (Appendix B), where the invasive European lineage first appeared in herbarium records ~150 years ago. Populations were selected to cover the entire known latitudinal range of *L. rufitarsis* in North America (36.5° to 43.6°, 789 km; Allen et al. 2015). Determination of lineage was made using chloroplast DNA and the methods of Saltonstall (2002) with modifications outlined in Kulmatiski et al. (2010). In many cases, populations of different *P. australis* lineages occurred in the same watershed but were rarely intermixed.

Sampling was conducted when galls were apparent during late summer (28 July – 30 August 2012). For each *P. australis* population, the proportion of stems with a *Lipara* gall was estimated by walking a single transect from the edge to interior and examining the three closest

stems every 1 m for the presence of a gall (150 stems total). To estimate the proportion of stems with a *L. rufitarsis* gall, all galls were collected during the survey, transferred to individual Ziploc bags, and placed in an environmental chamber (25 °C, 95% RH, 16:8 h light:dark) (see Allen et al. 2015). *Lipara* were identified to species based on gall and insect morphology, following Chvala et al. (1974). In this study, we focused only on *L. rufitarsis* because it was the only *Lipara* species widespread and abundant enough to test our predictions.

Common garden experiment

A complementary experiment was conducted at an established common garden at the University of Rhode Island, Kingston, RI (41.49° N, -71.54° W). We collected data from 74 populations of *P. australis* (28 native, 36 invasive, 10 Gulf), initiated with rhizome material collected from natal populations throughout North America, ranging in latitude from 26.6° to 46.1° (2,167 km) (Appendix B). Six native and seven invasive populations overlapped with those from the field study. The presence of the Gulf lineage in the common garden experiment represents a novel lineage to all three *Lipara* species, as their distributions do not overlap in nature (Allen et al. 2015). Rhizome material was planted in Metromix® soil (Sungro Horticulture, Agawam, Massachusetts) in 19 liter nursery pots. Plants were maintained in outdoor plastic pools filled with fresh water and were regularly fertilized with Mega Green organic fertilizer (Hydrolysate Company of America LLC, Isola, Mississippi). Because we removed panicles before seeds dehisced, only clonal rhizomatous growth occurred in the garden. Thus, it was not possible for the plants to evolve in response to the local environment. Consequently, any variation among common garden populations was expected to be due to genetic differences that originated in the natal location. Furthermore, by growing the plants for at

least two years prior to the start of our study, maternal effects that might drive differences in herbivory were minimized.

To assess herbivory of *L. rufitarsis* on *P. australis* populations under homogenous environmental conditions, we first removed all galled stems from the common garden in the winter of 2012-2013. We then seeded the garden on 18 April 2013 with 800 *L. rufitarsis* galls, sourced from an invasive *P. australis* population 8 km from the common garden (41.38° N, -71.51° W, Appendix B). The collected galls were evenly spread throughout the common garden, at a rate of ~1 gall per pot. *L. rufitarsis* were left to naturally emerge, mate, and select stems for oviposition. Plants and galls were allowed to develop naturally over the year.

The proportion of stems infested with *Lipara* per *P. australis* source population was quantified by inspecting each senesced stem for the presence of a gall during April 2014 (the year after the garden was seeded with *L. rufitarsis*). Each population was represented by 11.3 ± 1.0 pots (mean ± S.E.; range: 1 - 38, n = 74) and the number of stems examined per population averaged 119.8 ± 12.9 (range 10 - 432). All galls were collected and inhabitants reared in the laboratory to determine *Lipara* species identity.

To investigate the proximal factors that affect *L. rufitarsis* herbivory, we collected data on *P. australis* stem characteristics during the period when adult female *L. rufitarsis* were selecting plants for oviposition, 25 May to 10 June 2013 (based on Chvala et al. 1974). Stem density, height and diameter were the traits quantified, selected because they are known to influence oviposition and performance of gall-forming herbivores (e.g., Prado and Vieira 1999; Santos et al. 2008), including *Lipara* (De Bruyn 1994; Blossey 2014). The number of stems per pot were counted and converted to number/m^2. Stem height (measured from the base to the highest point of the stem) and stem diameter (measured at the first internode above the soil using

digital calipers) were obtained for a single randomly selected stem in each pot. We set a minimum criterion of three replicate pots for a population (mean of 15.6 ± 1.5 pots per population, n = 1,060) to be included in analyses. Therefore, the final data set consisted of 68 *P. australis* populations (24 native, 35 invasive, 9 Gulf).

Data analysis

Field survey

We tested whether latitudinal gradients in the proportion of stems galled by *L. rufitarsis* were present and whether they differed between the native and invasive *P. australis* lineages. We used a generalized linear model (GLM) with binomial distribution of errors, weighted by the total number of stems examined per population, and included population latitude and lineage as explanatory variables. A quadratic term (latitude^2) was also included to evaluate whether the relationship between the proportion of stems with galls and latitude was nonlinear. Possible interactions between lineage and latitude and lineage and latitude^2 were also tested, as they were deemed to be potentially important based on previous work with *P. australis* (Cronin et al. 2015, Bhattarai et al. in review). A combination of quantile-quantile plots and Cook's D were used to identify potential outliers and influential populations; although, none existed.

We used Akaike's Information Criteria corrected for finite sample size (AICc) to select the most informative model (Burnham and Anderson 2010). Candidate models were constructed from the full model (lineage, latitude, latitude^2, and the interactions between lineage and latitude and lineage and latitude^2) using all possible combinations of the variables, but with the restriction that interaction terms could only be included if their main effects were also present in the model. Candidate models were ranked by AICc from lowest to highest value and models with a ΔAICc value (= $AICc_i$ - $AICc_{min}$) of ≤ 2 were deemed to have substantial support (Burnham

and Anderson 2010). We also report the AICc weights which indicate the weight of evidence (as a proportion) in favor of model *i* being the best model given the set of candidate models. Finally, if the analysis indicated a significant latitude effect, we subsequently performed separate GLMs for each lineage to characterize relationships between the proportion of stems galled and latitude. Goodness of fit is reported as 1-(residual deviance/null deviance), which is comparable in interpretation to the coefficient of determination (R^2) for linear models (Menard, 2000).

Common garden experiment

Using the same GLM and AICc model selection approach as above, we tested whether the proportion of stems with a gall was influenced by *P. australis* lineage, source latitude, and stem characteristics during the *L. rufitarsis* oviposition period. Thus, our full model included lineage, latitude, latitude^2, stem density, height, and diameter at oviposition, as well as interactions between lineage and each of the other explanatory variables. We identified potential outliers and influential populations using the methods described previously. Three data points were removed from the analyses (one from each lineage, see Appendix S2). For each continuous explanatory variable present in the best fit model, we again performed separate GLMs for each *P. australis* lineage to characterize relationships with the proportion of stems galled. Finally, for each explanatory variable in the AICc top models across all analyses, we reported effect sizes (i.e., proportional differences in means or strength of relationship slopes) (Burnham and Anderson 2010). All analyses were performed in R 3.2.0. (R Development Core Team 2015) using the MuMIn package (Barton 2016).

RESULTS

Field survey

Variation in the proportion of stems galled by *L. rufitarsis* in the field was best explained by a single model (AICc = 325.7, R^2 = 0.674, AICc weight = 1.0). This was the full model, including lineage, latitude, latitude^2, and the interactions between lineage and latitude and lineage and latitude^2 as influential explanatory variables. In the field, the proportion of stems galled by *L. rufitarsis* on native *P. australis* populations (0.29 ± 0.04, mean ± SE) was five times higher than invasive *P. australis* populations (0.06 ± 0.02) (Fig. 3.1a). The relationship between the proportion of stems galled by *L. rufitarsis* and latitude depended upon *P. australis* lineage (i.e., the lineage × latitude and lineage × latitude^2 interactions were present in the best fit model). The relationship between the proportion of stems galled for the native lineage and latitude (based on a separate GLM for this lineage only) was nonlinear but increased from 0.27 at the southernmost site to 0.37 at the northernmost site (latitude^2: z = 4.30, R^2 = 0.134, P < 0.001, Fig. 3.1a). Conversely, the proportion of stems galled for the invasive lineage showed no relationship with latitude (z = 1.78, R^2 = 0.028, P = 0.075, Fig. 3.1a).

Common garden experiment

Like the field survey data, variation in *L. rufitarsis* herbivory was best explained by a single model, which included all terms except the lineage × stem height interaction (AICc = 568.9, R^2 = 0.721, AICc weight = 0.95). The average proportion of stems with a *L. rufitarsis* gall was only slightly higher on the native (0.42 ± 0.04) than invasive (0.41 ± 0.03) lineage of *P. australis*, but both were more than two times higher than on the Gulf lineage (0.17 ± 0.04) (Fig. 3.1b). As with the field survey, the effects of lineage on the proportion of stems with galls varied

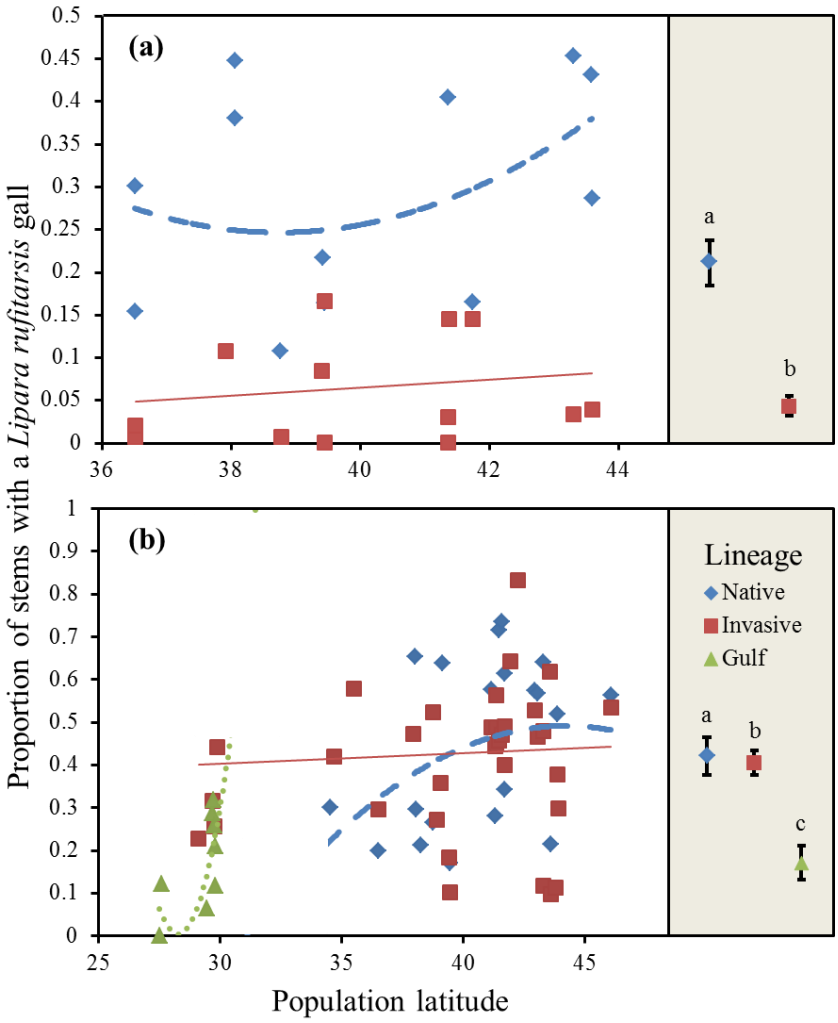

Figure 3.1. Relationship between the proportion of stems galled by *Lipara rufitarsis* and latitude for populations of the native, invasive, and Gulf *Phragmites australis* lineages in the (a) field survey and (b) common garden experiment. Regression lines are fit using parameter estimates from separate general linear models for each lineage (solid = invasive, dashed = native, dotted = Gulf) or from least-squares regression for nonlinear relationships. Thick lines denote significant relationships between the proportion of stems galled and population latitude ($P < 0.05$; see Appendix B). Symbols in the shaded portion of the figure depict the mean (± SE) proportion of stems galled for each lineage independent of latitude. Different lowercase letters indicate significant differences between means ($P < 0.05$).

generally positive correlations with latitude (latitude^2: $z = 3.41$, $R^2 = 0.050$, $P = 0.001$ and $z = 2.34$, $R^2 = 0.224$, $P = 0.019$, respectively, Fig. 3.1b, Appendix B). The proportion of stems galled increased from 0.23 to 0.48 from the southern to the northern end of the native lineage distribution (1,281 km). Moreover, the proportion of stems galled increased over three-fold

across the latitudinal range of the Gulf lineage; however, this gradient spanned just 2.3° latitude (260 km). In contrast, no relationship was detected between the proportion of stems galled and latitude for the invasive lineage ($z = 1.23$, $R^2 = 0.004$, $P = 0.218$, Fig. 3.1b).

Stem characteristics during the *L. rufitarsis* oviposition period were very important in explaining the proportion of stems with galls per source population. First, *L. rufitarsis* herbivory was strongly negatively correlated with mean stem height, regardless of *P. australis* lineage ($z = -15.85$, $R^2 = 0.338$, $P < 0.001$, Fig. 3.2a, Appendix B). The proportion of stems galled increased four-fold from the tallest to shortest populations at the time of oviposition in the common garden, the largest effect size of the experiment. Second, basal stem diameter was weakly positively correlated with the proportion of stems galled but the slope of the relationship depended on *P. australis* lineage (lineage × stem diameter interaction in the best fit model). The correlation was steeper for the Gulf ($z = 3.51$, $R^2 = 0.398$, $P < 0.001$) than native ($z = 3.36$, $R^2 = 0.049$, $P = 0.001$) and invasive ($z = 3.75$, $R^2 = 0.039$, $P < 0.001$) lineages, increasing 156%, 43% and 47% over the range of stem diameters for each lineage, respectively (Fig. 3.2b, Appendix B). Third, the native lineage exhibited a positive correlation ($z = 5.15$, $R^2 = 0.115$, $P < 0.001$), the Gulf lineage a negative correlation ($z = -3.13$, $R^2 = 0.296$, $P = 0.002$), and the invasive lineage exhibited no correlation ($z = 1.51$, $R^2 = 0.006$, $P = 0.131$) between stem density and the proportion of stems with galls (Fig. 3.2c, Appendix B). The proportion of stems galled increased by 58% over the range of native stem densities, and decreased by 56% over the range of Gulf stem densities.

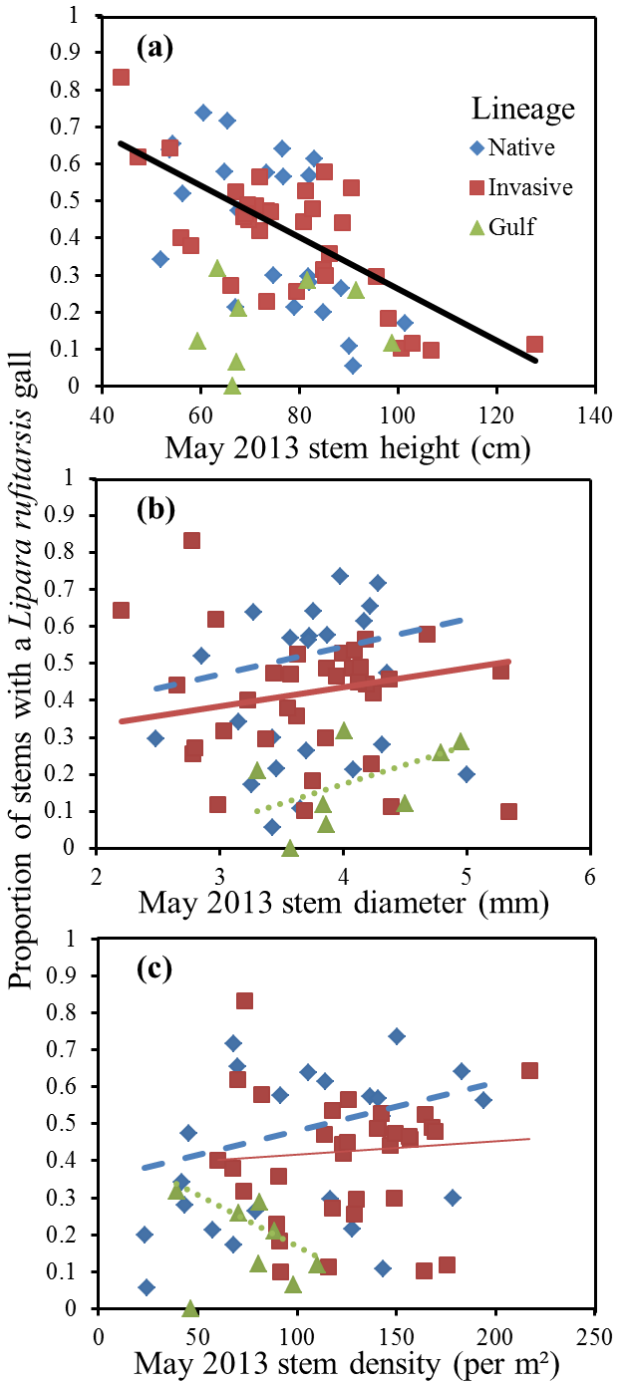

Figure 3.2. Relationship between the proportion of stems galled by *Lipara rufitarsis* and (a) stem height (cm), (b) stem diameter (mm), and (c) stem density (per m^2) during the *L. rufitarsis* oviposition period for native, invasive and Gulf lineages of *Phragmites australis* in the common garden experiment. Regression lines are fit using parameter estimates from general linear models for each stem characteristic. Individual lines for each lineage (solid = invasive, dashed = native, dotted = Gulf) are shown if the stem characteristic had a significant interaction with lineage in the best fit model. Thick lines denote significant relationships between *L. rufitarsis* herbivory and stem characteristics ($P < 0.05$; see Appendix B).

Because stem characteristics during *L. rufitarsis* oviposition were important predictors of the proportion of stems galled, we examined how each stem characteristic varied with respect to lineage, latitude (and the quadratic latitude^2), and their interaction using analysis of covariance (ANCOVA). Pairwise differences among *P. australis* lineages were assessed with a Tukey's test if lineage was significant in the ANCOVA. Stem density differed among *P. australis* lineages ($F_{2,59}$ = 5.15, P = 0.009, Table 3.1). It was higher for the invasive (124.9 ± 6.2) than native (100.0 ± 10.5; P = 0.046) and Gulf (84.3 ± 10.5) lineages, which were not significantly different from one another (P = 0.660). Stem height ($F_{2,59}$ = 0.79, P = 0.458, Table 3.1) and stem diameter ($F_{2,59}$ = 0.43, P = 0.653, Table 3.1) did not differ among *P. australis* lineages. Stem characteristics did not vary with latitude of origin of the *P. australis* populations (P > 0.05 for all variables).

Table 3.1. Mean (± SE) stem height (cm), diameter (mm) and density (per m^2) for each *Phragmites australis* lineage in the common garden. Different lowercase letters indicate significant differences between lineage means for each stem characteristic (P < 0.05).

	***Phragmites australis* lineage**		
	Native	**Invasive**	**Gulf**
Stem height (cm)	73.50^{a} ± 2.77	74.97^{a} ± 2.30	73.20^{a} ± 4.68
Stem diameter (cm)	3.71^{a} ± 0.11	3.61^{a} ± 0.11	3.93^{a} ± 0.25
Stem density (per m^2)	99.96^{a} ± 10.50	124.88^{b} ± 6.16	84.28^{a} ± 10.50

DISCUSSION

The native lineage of *P. australis* exhibited a positive correlation in the field between herbivory from a specialist stem gall-fly (*L. rufitarsis*) and latitude, whereas no relationship with latitude was detected for the invasive lineage. Because of the non-parallel latitudinal gradients

between the native and invasive lineages, the strength of local enemy release of invasive *P. australis* from *L. rufitarsis* was highest at northern latitudes. Latitudinal gradients observed in the field were reflected in the common garden experiment, suggesting an underlying genetic basis to these biogeographic patterns. Moreover, stem characteristics (height, diameter, density) measured during the *L. rufitarsis* oviposition period were key determinants of herbivory, whereas there was very little difference in herbivory between the native and invasive *P. australis* lineages, with the only difference among lineages being the substantially lower herbivory on the Gulf lineage. This result suggests that the strong difference in the proportion of stems galled between native and invasive *P. australis* lineages observed in the field was not genetically based but rather driven by the effects of local environmental conditions on plant growth and the subsequent response of *L. rufitarsis*. Along with the studies by Cronin et al. (2015) and Bhattarai et al. (in review) which focused on generalist herbivores of *P. australis*, our study suggests that, regardless of degree of herbivore specificity, genetically based latitudinal gradients in herbivory and qualitative differences in those gradients between sympatric native and invasive plant taxa may be common phenomena. These biogeographic patterns can have important implications for understanding successful species invasions.

Non-parallel latitudinal gradients in *Lipara rufitarsis* herbivory

Although evidence to date is limited to only one plant system, *P. australis*, this study lends support to the idea that local enemy release is strongly dependent on biogeography (see also Cronin et al. 2015; Bhattarai et al. in review). Our prediction that native and invasive *P. australis* lineages exhibit non-parallel latitudinal gradients in the proportion of stems with *L. rufitarsis* galls was upheld. In the field, we found that the proportion of stems with galls in native *P. australis* populations increased by 37% from our southernmost to our northernmost

populations, whereas there was no relationship between the proportion of stems galled and latitude for the invasive lineage. These non-parallel gradients between native and invasive lineages were reflected in the common garden experiment. Because the proportion of stems galled on the invasive lineage did not vary with latitude, the difference in herbivory between the two lineages diverged with increasing latitude. The result was that local enemy release was stronger in the north than south. Due to the controlled environment in the common garden, these biogeographic patterns are genetically based rather than the result of phenotypic plasticity, thus supporting our third prediction.

Cronin et al. (2015) and Bhattarai et al. (in review) have previously described biogeographic heterogeneity in the strength of local enemy release of invasive *P. australis* in the field and common garden, respectively. These studies focused on generalist herbivores (the mealy plum aphid, *Hyalopterus pruni* [Geoffroy], or the fall armyworm, *Spodoptera frugiperda* [J. E. Smith]) or the combined effects of entire herbivore guilds (leaf chewers, internal stem feeders), whereas the current study focused on an obligate specialist of *P. australis*. We expected that local adaptation by native and invasive *P. australis* to a specialist herbivore would be more likely than to generalist herbivores. However, for the native lineage, *H. pruni* exhibited a negative genetically based latitudinal gradient, *L. rufitarsis* a positive genetically based latitudinal gradient, and *S. frugiperda* showed no evidence of a gradient. Interestingly, the invasive lineage only exhibited a negative genetically based latitudinal gradient for the *H. pruni* aphids. These findings concur with those of Anstett et al. (2014) and Kim (2014) who found no clear distinction between specialist and generalist herbivores in the likelihood that their host plants evolved a genetically based latitudinal gradient in susceptibility to attack. Interestingly, *L. rufitarsis*, *H. pruni*, the guild of leaf chewers, and the guild of internal stem feeders all exhibited

non-parallel latitudinal gradients in herbivory in which a gradient was evident for the native lineage but not the invasive lineage. Ultimately, this results in a tremendous amount of spatial heterogeneity in local enemy release or biotic resistance for the invasive lineage of *P. australis*.

We offer some possible mechanisms which could lead to non-parallel latitudinal gradients in *L. rufitarsis* herbivory on invasive and native *P. australis* lineages. First, *Lipara* have only been present in North America for less than 100 years (Sabrosky 1958; Tewksbury et al. 2002), meaning all *P. australis* lineages in North America have had approximately the same period of time to evolve latitudinal gradients in response to *Lipara* herbivory. However, because the native lineage has been present in North America for millennia, it is possible that there are pre-existing latitudinal gradients in some plant traits which may be important in determining outcomes of plant-herbivore interactions. For example, leaf tissue nitrogen content, a key nutrient for many herbivores (Mattson Jr. 1980), increased with latitude for native but not invasive *P. australis* in the field and garden (Cronin et al. 2015; Bhattarai et al. in review). Unfortunately, we did not quantify nitrogen content in this study, so were unable to assess whether this gradient is related to *L. rufitarsis* herbivory. Second, local adaptation to herbivores may be more likely for native *P. australis* populations, which are more isolated from one another and thus potentially experience less gene flow relative to invasive populations. However, this possibility is contradicted by Bhattarai et al. (in review), who observed a genetically based negative correlation between latitude and palatability to aphids for the invasive *P. australis* lineage. Third, a number of studies with replicate common gardens have found that latitudinal gradients in traits associated with plant-herbivore interactions are phenotypically plastic (Woods et al. 2012; Bhattarai et al. in review) and that invasive taxa are more plastic than native taxa (Richards et al. 2006; Davidson et al. 2011; Bhattarai et al. in review). Thus, expression of

latitudinal gradients may depend upon complex interactions between plant lineage and local environmental conditions and the patterns observed in this study may be altered under different common garden conditions.

To date, virtually nothing has been reported about the ecology of the Gulf lineage and its interactions with other species, and its introduction history and invasive status in the United States is currently unclear (Lambertini et al. 2012; Meyerson et al. 2016). Here, the positive latitudinal gradient associated with the Gulf lineage occurs over only 2.3° (260 km) latitude and thus may be a result of examining only a narrow range of latitudes, rather than an evolved relationship. Including a larger portion of the Gulf lineage range (e.g., from the Gulf states to Central America; Lambertini et al. 2012; Colin and Eguiarte 2016) would better elucidate the relationship between latitude and *L. rufitarsis* herbivory for this lineage.

Local enemy release for the invasive *Phragmites australis* lineage

Although it has long been argued that leaving behind coadapted natural enemies (i.e., the enemy release hypothesis) can facilitate invasions (Elton 1958; Keane and Crawley 2002), ultimately invasion success may depend on whether the non-native plant species can withstand the impact of herbivores in their new range (i.e., the local enemy release hypothesis; Zheng et al. 2012). In support of this hypothesis (our second prediction), we found the proportion of stems galled by *L. rufitarsis* was lower on the invasive than native *P. australis* lineage in the field. This result is consistent with previous studies involving *Lipara*, other herbivores, and *P. australis* in North America (Lambert and Casagrande 2007; Lambert et al. 2007; Park and Blossey 2008; Allen et al. 2015; Cronin et al. 2015; Cronin et al. 2016). Moreover, local enemy release of invasive plants has widespread support across a range of field and common garden studies in various systems (e.g., Agrawal et al. 2005; Parker and Gilbert 2007; Zheng et al. 2012). Because

of their strong effect on plant fitness through prevention of flowering (Lambert et al. 2007; Allen et al. 2015), local enemy release from *L. rufitarsis* is likely to have a significant negative impact on the native lineage relative to the invasive lineage (Cronin et al. 2016).

Despite the strong evidence for local enemy release of invasive *P. australis* from *L. rufitarsis* in the field, there was almost no difference (just 1%) in the proportion of stems galled between native and invasive *P. australis* in the controlled common garden. These data suggest that there is no genetic basis for the difference in *L. rufitarsis* herbivory between native and invasive lineages in our field survey. This finding is somewhat surprising because the invasive lineage has had a much longer history of association with all three *Lipara* species (both originate from Europe) than the native lineage and is therefore more likely to have evolved defenses against attack. Multiple other studies have previously documented contrasting results between field and common garden patterns of herbivory (Park and Blossey 2008; Woods et al. 2012; Hiura and Nakamura 2013), generally attributed to the variable influence of local environmental conditions in the field (i.e., phenotypic plasticity). Thus, the discrepancy between our field and garden studies suggests that the strong local enemy release observed in the field is likely the result of phenotypic plasticity and/or legacy effects (e.g., *Lipara* herbivory is historically higher in association with native than invasive populations in the field), rather than genetic differences between native and invasive *P. australis* lineages. As many studies have demonstrated that invasive taxa are more phenotypically plastic than native taxa (e.g., Richards et al. 2006; Davidson et al. 2011), including with *P. australis* (Bhattarai et al. in review), it is possible that the local enemy release we observed in the field is driven by a strong plastic response on the part of the invasive lineage.

Finally, the proportion of stems galled by *L. rufitarsis* on the Gulf lineage in the common garden was less than half that of the native and invasive lineages. The Gulf lineage has never interacted with *Lipara* due to their isolated distributions, thus any patterns in plant-herbivore interaction strength for this lineage are likely due to pre-existing adaptations to other herbivores or selection pressures rather than coevolution. Regardless, the strong local enemy release of the Gulf lineage (relative to the other two lineages) suggests that if their distribution were ever to overlap with *Lipara*, the Gulf lineage may have an advantage over other *P. australis* lineages. For many of the other common herbivores of *P. australis*, herbivory has generally been similar between the Gulf and invasive lineages (Cronin, J. T., Bhattarai, G. P., Allen, W. J., Meyerson, L. A., unpublished data). Currently, we have not identified the traits which confer such strong resistance to the Gulf lineage.

***Lipara rufitarsis* herbivory depends on stem characteristics**

Plant morphological traits have often been shown to be useful predictors of attack and damage by gall insects and herbivores in general (e.g., De Bruyn 1994; Prado and Vieira 1999; Santos et al. 2008). In support of our fourth prediction, we found that stem characteristics during the oviposition period of *L. rufitarsis* were strongly correlated with the subsequent proportion of stems galled. Most importantly, the proportion of stems galled was much higher in native and invasive source populations that had shorter and thicker stems. In contrast to our findings, De Bruyn (1994) demonstrated that *L. rufitarsis* females preferred to oviposit on thinner stems of the invasive lineage (in its native range), around 4-5 mm in diameter. However, the majority of stems in our study were 3-5 mm in diameter (Fig. 3.2b), thus we may not have covered a large enough range of stem diameters for such a negative correlation to become apparent (De Bruyn 1994). Neither of these stem characteristics differed between lineages. Thus, differences in

latitudinal gradients between the native and invasive lineage cannot be attributed to variation in stem height or diameter.

Host plant density is often cited as an important factor driving oviposition and herbivory of many gall-forming insects (e.g., Abrahamson et al. 1983; Cuevas-Reyes et al. 2004), and previous studies have found *Lipara* herbivory to be positively correlated (Blossey 2014) and unrelated (Allen et al. 2015) to stem density. In this study, we found that the proportion of stems galled by *L. rufitarsis* and stem density were positively correlated for the native lineage, negatively correlated for the Gulf lineage, and showed no correlation for the invasive lineage. Furthermore, stem density was higher for the invasive than native and Gulf lineages, and this type of dense clonal growth is commonly regarded as a trait of invasive taxa (Thompson et al. 1995; Liu et al. 2006). Taken together, these results suggest that for the native *P. australis* lineage, any competitive advantage gained through higher stem density may be negated by increased herbivory by *L. rufitarsis*. In contrast, the invasive lineage experiences no such trade-off between high stem density and the degree of herbivory, while increased stem density may even assist the Gulf lineage in escaping herbivory.

These stem characteristics are likely to be strongly influenced by the local environment. In nature, the native and invasive lineages in North America often occupy different microhabitats related to salinity, hydrology, disturbance, and nutrient availability (e.g., Vasquez et al. 2005; Holdredge et al. 2010; Price et al. 2014). For example, native *P. australis* populations may be more prevalent in nutrient-poor environments, where they are better able to compete (Holdredge et al. 2010). This environment could result in a higher proportion of short, stressed stems, thus making the native lineage more attractive to *L. rufitarsis* for oviposition (De Bruyn 1994). In a well fertilized and watered common garden, plants were unstressed and subjected to the same

environmental conditions. Therefore, lineage-specific patterns in the field (driven by microclimatic effects on stem characteristics) may be negated in a common garden.

Conclusions

We find that local enemy release of the invasive *P. australis* lineage from *L. rufitarsis* is likely a plastic response, driven by stem characteristics that are modified by local environmental conditions, rather than the result of genetic differences between native and invasive lineages. Latitudinal variation in the strength of the local enemy release is subsequently generated by local adaptation of the native but not invasive lineage along a latitudinal gradient. The result is non-parallel latitudinal variation in herbivory by *L. rufitarsis* such that the invasive lineage suffers proportionately less herbivory than the native lineage (i.e., greater local enemy release) at high than low latitudes. Geographic variation in local enemy release is widespread in *P. australis* for both generalist and specialist herbivores – the strength of release from the aphid *H. pruni*, the guild of leaf chewers and the guild of internal stem feeders all vary linearly with latitude (Cronin et al. 2015). Unfortunately, the *P. australis* system is the only one in which the biogeography of local enemy release has been explored. However, we suggest that herbivory of co-occurring native and invasive plant taxa with respect to latitude is likely to be dissimilar owing to many factors including different phylogenies, historical distributions, and coevolutionary histories with local herbivores. We suggest that geographic heterogeneity in herbivory of native and invasive plant taxa can result in corresponding heterogeneity in the establishment and/or spread of invasive plant species. On these grounds, we argue for a broader, biogeographic perspective to the study of invasive species. Moreover, because invasive species can evolve rapidly in response to environmental gradients (Bhattarai et al. in review; Li et al. 2015; Maron et al. 2004) and native and invasive species may differ in evolutionary trajectories, differences in local enemy

release and biotic resistance are likely to be transient. Thus, future studies in this area should investigate temporal as well as spatial variability in invasive-native plant species interactions. Finally, the majority of studies examining biogeographic variation in species interactions have focused on herbivory (Schemske et al. 2009). However, the ideas in this paper also apply to other interactions such as mutualisms, competition, and higher trophic level interactions, which remain unexplored using a biogeographic perspective in invasive-native systems.

CHAPTER 4
PLANT-SOIL FEEDBACKS, SPILLOVER AND COMPETITION BETWEEN NATIVE AND INVASIVE WETLAND PLANT SPECIES

INTRODUCTION

It is widely accepted that plant species possess the ability to influence community composition and function of soil biota, which in turn can impact fitness of the host plant species, a reciprocal interaction commonly referred to as a plant-soil feedback (PSF) (Ehrenfeld et al. 2005; Kulmatiski et al. 2008). The net impact of soil biota on their host plant depends on the balance between beneficial interactions involving nitrogen-fixing bacteria, mycorrhizal fungi and other mutualists against harmful interactions with soil-borne pathogens, parasites, and herbivores (Westover and Bever 2001; Klironomos 2002; Reinhart and Callaway 2006). PSFs are integral to plant community dynamics (van der Putten et al. 1993; Bever et al. 1997; Klironomos 2002; Wardle et al. 2004; Maron et al. 2011; van der Putten et al. 2013; Suding et al. 2013) and a well-supported prediction is that negative PSFs promote species coexistence, whereas positive PSFs lead to species dominance (Bever et al. 1997; Reynolds et al. 2003).

This prediction has clear implications for the success of invasive plants. For example, invasive plants could experience less positive or more negative PSFs relative to closely-related native species (i.e., weaker associations with mutualists or greater attack by local natural enemies), supporting biotic resistance of the native community (Elton 1958). In contrast, invasive plant species may generate more positive/less negative PSFs than closely-related native species (i.e., stronger associations with mutualists or escape from local natural enemies), potentially resulting in dominance for the invader. This latter scenario has fairly strong support from a number of empirical studies, meta-analyses and reviews (e.g., Klironomos 2002; Agrawal et al. 2005; Van Grunsven et al. 2007; Kulmatiski et al. 2008; MacDougall et al. 2011).

While it is clear that soil biota can directly impact host plant fitness, we know relatively little about their context dependency and particularly how PSFs interact with other important processes linked to species invasions such as species interactions, disturbance, and increased nutrient availability (Suding et al. 2013). For example, modeling and experimental studies have demonstrated that even relatively small PSFs can alter interspecific competitive ability (e.g., Marler et al. 1999; Bever 2003; Casper and Castelli 2007; Hodge and Fitter 2013), which is another key mechanism in determining the success of invasive species (see Gioria and Osborne 2014 for review). Furthermore, some invaders cultivate generalist soil biota that may also interact with native species, resulting in indirect effects of the invasive species mediated through PSF (i.e., pathogen/mutualist spillover, apparent competition/mutualisms) (Eppinga et al. 2006; Niu et al. 2007; Mangla et al. 2008). Moreover, co-occurring native species may be inhibited by soil biota even after removal of the invader (i.e., soil legacies) (Eviner and Hawkes 2008; Corbin and D'Antonio 2012). To date, it is unknown if spillover and soil legacies differ between closely-related native and invasive taxa, which may have important implications for understanding drivers of invasion success and approaches necessary for successful restoration of invaded communities.

Anthropogenic nutrient deposition is a major component of global environmental change and a facilitating factor of many plant invasions (Vitousek et al. 1997; Dukes and Mooney 1999). Nutrient availability can alter competitive interactions (Wilson and Tilman 1993), activity of plant mutualists and pathogens in the soil (Johnson et al. 2008), and thus the direction and magnitude of PSFs (Manning et al. 2008). The interaction between PSFs, interspecific competition and nutrient availability could differ among native and invasive taxa, ultimately impacting the resistance/susceptibility of native communities to invasions. Currently, there are

few studies that have compared the interactive effects of soil biota, interspecific competition, and nutrient availability between native and invasive plant taxa (but see Larios and Suding 2015).

The goal of this study was to investigate the effects of soil biota, interspecific plant competition, and nutrient availability on the relative performance (biomass production, biomass allocation) of the native and two invasive lineages of common reed (*Phragmites australis* [Cav.] Trin. ex Steudel) (Poaceae) in North America. In a greenhouse experiment, we grew replicates of three populations each of the three lineages in pots containing live or sterilized soil inoculum from the rhizosphere of the *P. australis* population. To examine the interaction between PSFs, interspecific competition and nutrient availability, and possible spillover effects of soil biota onto the native plant community, we grew *P. australis* at two nutrient levels and with or without native smooth cordgrass (*Spartina alterniflora* Loisel.), a common co-inhabitant of marshes occupied by *P. australis*. We tested the following predictions: 1) invasive *P. australis* lineages experience more positive PSFs than the native lineage; 2) spillover of soil biota from invasive lineages has more negative effects on *S. alterniflora* than soil biota from the native lineage; 3) the direction and strength of PSFs and spillover depends on the presence of an interspecific competitor and nutrient availability; 4) invasive lineages of *P. australis* possess stronger interspecific competitive ability than native lineages and *S. alterniflora*; 5) PSFs and nutrient availability alter interspecific competition between *P. australis* and *S. alterniflora*; 6) invasive *P. australis* lineages respond more positively to increased nutrient availability than the native lineage and *S. alterniflora*; and 7) plant responses to nutrient availability are influenced by soil biota and interspecific competition.

MATERIALS AND METHODS

Study organisms

Phragmites australis is a model organism for studying plant invasions (Meyerson et al. 2016) and is one of the most widely distributed plants in the world, occurring in coastal marshes, inland lakes and rivers, deserts, mountains, and metropolitan areas (Marks et al. 1994; Clevering and Lissner 1999). Multiple lineages of *P. australis* grow sympatrically in North America (Saltonstall, 2002; Meyerson et al. 2009; Lambertini et al. 2012; Meyerson et al. 2012; Meyerson and Cronin 2013). The native lineage is endemic to North America and consists of at least fourteen different haplotypes (Saltonstall 2002; Meadows and Saltonstall 2007; Vachon and Freeland 2011). An invasive lineage of *P. australis* from Europe has spread aggressively in wetlands of North America over the last 150 years (Chambers et al. 1999; Saltonstall 2002; Howard et al. 2008; Meyerson et al. 2012; Meyerson and Cronin 2013). This European lineage is comprised of mostly a single haplotype (*M*) and forms large, dense, monospecific populations which negatively impact hydrology, biogeochemical processes, ecosystem function, native plant diversity, and habitat quality for fauna (Meyerson et al. 2000; Saltonstall 2002; Gratton and Denno 2005; Meyerson et al. 2009). An additional lineage (known as Gulf) is common and widely distributed along the Gulf of Mexico and west to California (Hauber et al. 2011; Lambertini et al. 2012; Meyerson et al. 2012). This lineage is likely a recent arrival from Mexico or Central America, where it is native (Colin and Eguiarte 2016). Although its mode of introduction in North America is largely unknown, we classify it as invasive (following Richardson et al. 2000a) owing to its rapidly-growing populations (Bhattarai and Cronin 2014) and the speed with which it spread from the Gulf to the West Coast (Meyerson et al. 2012).

The diversity and function of the *P. australis* microbiome is presently being investigated (see Kowalski et al. 2015 for review) and a number of recent studies have described distinct oomycete, archaea, and bacteria communities from rhizosphere soil of native and European *P. australis* lineages in North America (Nelson and Karp 2013; Crocker et al. 2015; Yarwood et al. in press; Bowen et al. in review). These divergent microbial communities suggest that the net impact of soil biota may also differ among and within *P. australis* lineages. However, virtually all studies to date have focused on describing community structure of soil biota, whereas the direction and magnitude of their impacts on each *P. australis* lineage remain relatively unknown. The exception is the study by Crocker et al. (2015), in which it was demonstrated that virulence of some *Pythium* spp. oomycetes differed between native and European lineages. To date, virtually nothing has been reported about the ecology, trophic interactions, or microbial community of the Gulf lineage (but see Chapter 3; Bowen et al. in review).

Greenhouse experiment design

We conducted a greenhouse experiment to examine the interactive effects of soil biota, interspecific competition, and nutrient availability on daily biomass production and biomass allocation to belowground tissues (rhizomes and roots) of the three main lineages of *P. australis* in North America and a native competitor, *S. alterniflora*. The experimental design consisted of all four treatments – soil biota, presence of an interspecific competitor, nutrient level, and *P. australis* lineage – being fully crossed (thirty-six total treatment combinations) and replicated among three distinct *P. australis* populations within each lineage (Table 4.1).

Treatment 1) *Soil inoculum* – Live or sterilized soil inoculum collected in the field from the rhizosphere of each *P. australis* population was added to each pot to introduce soil biota.

Table 4.1. List of *Phragmites australis* field populations used for the greenhouse experiment.

Population name, state (ID code)	Latitude	Longitude	Lineage	Status
Palm Canyon Road, CA (PCN)	33.83	-116.62	Native	Endemic
Little Caliente Hot Springs, CA (LCN)	34.54	-119.62	Native	Endemic
Mackay Island, NC (NCN)	36.51	-75.95	Native	Endemic
East Cameron, LA (ECM)	29.77	-93.29	European	Invasive
I-40, AZ (I40M)	34.72	-114.49	European	Invasive
Mackay Island, NC (NCM)	36.51	-75.95	European	Invasive
Okeeheelee Park, FL (FLI)	26.65	-80.16	Gulf	Invasive
Intracoastal City, LA (ICI)	29.78	-92.20	Gulf	Invasive
Creole, LA (CRI)	29.83	-93.11	Gulf	Invasive

each *P. australis* population was visited during 25 March to 12 April 2015 and bulk rhizosphere soil (~15 kg total) was collected from five locations along a transect from the population edge to interior by excavating clumps of *P. australis* rhizomes (depth 0-50 cm), discarding loose soil, and shaking root- and rhizome-adhered soil into Ziploc bags. Soil was transported in an ice chest to the greenhouse within 48 hours. After thoroughly homogenizing the soil (by hand), one half of the soil was sterilized using an autoclave (134 °C at 100 kPA for 45 minutes).

Pots (1 L) were filled with 120 g of live or sterile soil inoculum combined with sterile (autoclaved) sand. To minimize nutrient flushes that can occur following soil sterilization (Troelsta et al. 2001) and the effects of varying abiotic properties associated with the different soil sources, we used a low inoculum:sand ratio (10% of total soil weight) and included a nutrient addition treatment (see below). This soil inoculation method has been used often to test for effects of soil biota on host plant species (e.g., Brinkman et al. 2010; Maron et al. 2014).

Treatment 2) *Interspecific competition* – Pots were planted with either *P. australis*, *S. alterniflora*, or both species combined. *S. alterniflora* was selected as a standardized native competitor because it is a dominant plant in many coastal marshes where it also co-occurs with *P. australis* (Bertness 1991; Meyerson et al. 2000; Medeiros et al. 2013) and even shares some

pathogen species (Li et al. 2014). To control for intraspecific genetic variation within *S. alterniflora*, we obtained plants as 5 cm plugs propagated from a single clone from Sarasota, FL (27.29° N, -82.53° W; Aquatic Plants of Florida, Sarasota, FL). *P. australis* was propagated using 5-15 g rhizomes sourced from populations which had been maintained in a common garden for at least three years (see Bhattarai 2015, Bhattarai et al. in review), minimizing maternal effects on *P. australis* competitive ability and response to microbes. Before planting, rhizomes and roots of both plant species were surface sterilized by submersion in 10% sodium hypochlorite for five minutes to remove epiphytic microbes (e.g., Parepa et al. 2013). Planting was staggered over a six week period during 1 April to 12 May 2015 because of the travel required to collect bulk soil, the large number of replicates, and the replacement of some rhizomes and plugs which did not establish successfully. Because plants were given so long to grow (226 ± 0.4 days, mean ± S.E.), any minor variation in initial rhizome/plug size was considered to be relatively unimportant to final biomass measurements.

Treatment 3) *Nutrient availability* – Nutrient levels were manipulated to represent nutrient-rich and nutrient-poor environments. Each nutrient-poor pot had 200 mL of Ferti-lome root stimulator and plant starter solution (Ferti-lome, Bonham, TX) (4% N, 10% P, 3% K; diluted at 1:76) added on 9 June, 23 July, and 2 September 2015. Pots assigned to the nutrient-rich treatment also received the Ferti-lome root stimulator plus an additional 10 g of Osmocote® Plus (Scotts, Marysville, OH) added on 23 July 2015, a high strength (19% N, 6% P, 12% K) and extended release (four months) fertilizer. This treatment represented an environment experiencing anthropogenic nutrient enrichment.

Nine distinct populations of *P. australis* (three of each lineage) were used for the experiment (Table 1). Populations were selected to represent a broad geographic distribution of

the three main *P. australis* lineages in North America and to use populations adapted to a southern climate comparable to the conditions in our greenhouse. Thus, the populations and soil inocula originated from southern California (2 native, 1 European), Louisiana (1 European, 2 Gulf), Florida (1 Gulf), and North Carolina (1 native, 1 European). It was not possible to represent all three lineages from each location because southern California is the only location in North America where they all co-occur (Meyerson et al. 2012). We planted ten replicates of the twelve treatment combinations for each of the nine *P. australis* populations, resulting in a total of 1,080 pots. Seventy-one pots were removed from the experiment and analyses due to mortality of replacement plantings (7% of total pots), and thirty-five other pots initially planted with two species were transferred to the appropriate single-species treatment when establishment of one species was unsuccessful (3%). Both these factors resulted in a slightly unbalanced experimental design.

Plants were grown in a greenhouse located at Louisiana State University (30.36° N, -91.14° W) with pots arranged in a randomized blocked design with five blocks to account for possible gradients in environmental conditions within the greenhouse. All pots were maintained in individual 2 L plastic trays with a constant supply of water to replicate wetland conditions. Herbivores were excluded by regular foliar spray applications of the low residue pesticide Safer® Soap (Safer®, Lititz, PA).

Data collection

Data collection and harvesting was completed from 5 to 13 December 2015. Plants were still green and healthy at this time and were considered to still be growing because they were still producing new stems and leaves and had not yet reached the flowering stage. Above and belowground biomass were harvested for each species from each pot, dried to constant mass, and

weighed to the nearest 0.1 g. Total biomass was adjusted by the number of growing days between planting and harvest to account for the staggered planting. Because no plants in our experiment produced a panicle, daily biomass production (i.e., clonal growth) was considered the most appropriate measure of fitness. To assess how our treatments influence the allocation of biomass to above and belowground structures, we also calculated the proportion of total biomass each plant allocated to belowground tissues. Biomass allocation was examined because variation between treatments would represent a plastic response of the plant to local conditions and may provide insight into allocation strategies which could alter competitive ability, responses to nutrient availability, or the frequency and strength of interactions with soil biota.

Data analysis

To examine how each dependent variable (total biomass produced per day, proportion of biomass allocated to belowground tissues) for each plant species (*P. australis, S. alterniflora*) was influenced by *P. australis* lineage, soil biota, presence of an interspecific competitor, and nutrient availability, we used Akaike's Information Criteria corrected for finite sample size (AICc) to select the most informative mixed-effects model from a set of candidate models (Burnham and Anderson 2010). The full model included the variables *P. australis* lineage (native, European, Gulf), live/sterile soil inoculum, presence/absence of an interspecific competitor, high/low nutrient availability, and all two-, three-, and four-way interactions as fixed effects (fifteen total variables). *P. australis* population and greenhouse block were included as random effects. Daily biomass production was square root transformed to normalize data distributions. Candidate models were constructed from the full model using all possible combinations of the variables, but with two restrictions. First, interaction terms could only be included if their main effects were also present in the model. Second, the random effects were

retained in every model combination because without this underlying structure the model design would be pseudoreplicated. Cook's D and quantile-quantile plots were used to identify potentially influential data points. However, in no case did removal of these data points qualitatively change model conclusions, thus we retained them in analyses.

We ranked candidate models from lowest to highest AICc value and models with a ΔAICc value (= $AICc_i$ - $AICc_{min}$) of ≤ 2 were deemed to have substantial support (Burnham and Anderson 2010). We also report AICc weights which indicate the proportional strength of support for model *i* being the best model. We estimated least-squares means (back-transformed for daily biomass production) based on the best fit model for each dependent variable and focused on effect sizes (i.e., proportional differences in means) in our interpretation of statistical analyses (Burnham and Anderson 2010). Finally, the maximum likelihood method was used for model selection and the restricted maximum likelihood method was used to estimate each best fit model (Zurr et al. 2009). For brevity, only results for models with AICc weight ≥ 0.30 are reported (i.e., the top model for each dependent variable). All analyses were performed in R 3.2.0. (R Development Core Team 2015) using the MuMIn package (Barton 2016).

RESULTS

Total daily biomass production

AICc model selection strongly supported the inclusion of live/sterile soil inoculum, presence/absence of an interspecific competitor, and high/low nutrient availability as influential explanatory variables in models explaining variation in *P. australis* daily biomass production. Four candidate models received adequate support (ΔAICc ≤ 2) and all included these same three main effects and interactions between them (cumulative AICc weight = 1). The top model (AICc = -863.9, AICc weight = 0.436) included only the main effects and had more than two times the

support of the other three models (second top model: AICc = -862.4, ΔAICc = 1.48, AICc weight = 0.207, see Appendix C for additional details). For *S. alterniflora,* variation in daily biomass production was best explained by *P. australis* lineage, live/sterile soil inoculum, presence/absence of an interspecific competitor, high/low nutrient availability, and the lineage × soil inoculum, lineage × nutrient availability, interspecific competitor × soil inoculum, and interspecific competitor × nutrient availability interactions (the top model: AICc = -1245.2, AICc weight = 0.711; Appendix C). The second top model (AICc = -1243.4, ΔAICc = 1.80, AICc weight = 0.289) also included all of these variables but had less than half the support of the top model.

Average daily biomass production was 11% lower for *P. australis* grown in pots containing live than sterile soil inoculum (Fig. 4.1A), regardless of lineage, presence of an interspecific competitor, or nutrient availability (i.e., no influential interactions in the top model). In contrast, the effect of soil inoculum on *S. alterniflora* daily biomass production depended upon the presence/absence of *P. australis* as a competitor (i.e., interspecific competitor × soil inoculum interaction) as well as the *P. australis* lineage the soil inoculum was sourced from (i.e., lineage × soil inoculum interaction). When *S. alterniflora* was grown alone, daily biomass production was 14% lower in pots with live than sterile soil inoculum (Fig. 4.2A). Interestingly, when competing with *P. australis*, *S. alterniflora* plants in live soil inoculum had 5% higher daily biomass production than those in sterile inoculum. Moreover, daily biomass production of *S. alterniflora* decreased by 14% in pots containing live soil inoculum from the two invasive lineages, but increased by 11% in live soil inoculum from the native lineage (Fig. 4.3A).

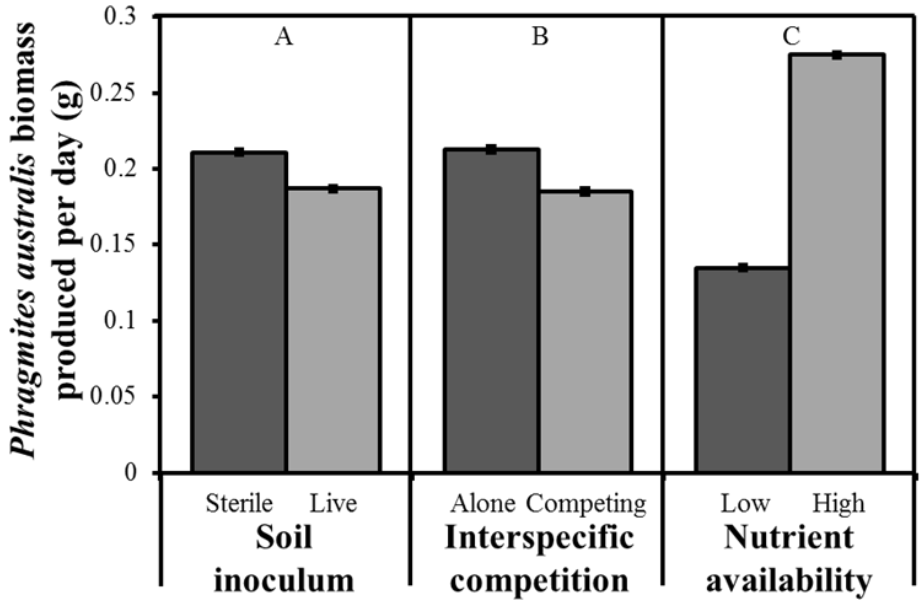

Figure 4.1. Mean (± S.E.) biomass produced per growing day (g) of *Phragmites australis* under various treatments: A) live or sterilized soil biota inoculum, B) alone or competing with *Spartina alterniflora*, and C) high or low nutrient availability. Error bars are obscured due to their small size.

Interspecific competition reduced *S. alterniflora* daily biomass production by 52% in live soil inoculum and 60% in sterile soil inoculum nutrient-rich pots (Fig. 4.2A). The impact of interspecific competition also depended upon nutrient levels, decreasing *S. alterniflora* biomass by 52% and 58% in nutrient-poor and nutrient-rich pots, respectively (Fig. 4.2B). In contrast, competition with *S. alterniflora* only reduced daily biomass production of *P. australis* by 13% relative to when grown alone (Fig. 4.1B).

Biomass production doubled (104% increase) for *P. australis* grown in nutrient-rich than nutrient-poor pots (Fig. 4.1C), regardless of lineage, live/sterile soil inoculum, or presence/absence of a competitor. Nutrient availability had an even stronger effect on daily biomass production of *S. alterniflora*, increasing 176% and 143% in nutrient-rich pots when grown alone and with *P. australis* as a competitor, respectively (Fig. 4.2B). Finally, in nutrient-

poor pots, differences in daily biomass production of *S. alterniflora* in pots with soil inoculum from different *P. australis* lineages were small (< 3%, range of 0.055 to 0.057 ± 0.0003 g, least-squares mean ± S.E.). However, in nutrient-rich pots, *S. alterniflora* grown in pots with soil inoculum from the invasive lineages of *P. australis* had 21-24% higher daily biomass production (European: 0.154 ± 0.0003 g; Gulf: 0.157 ± 0.0003 g) than pots with soil inoculum from the native lineage (0.127 ± 0.0003 g).

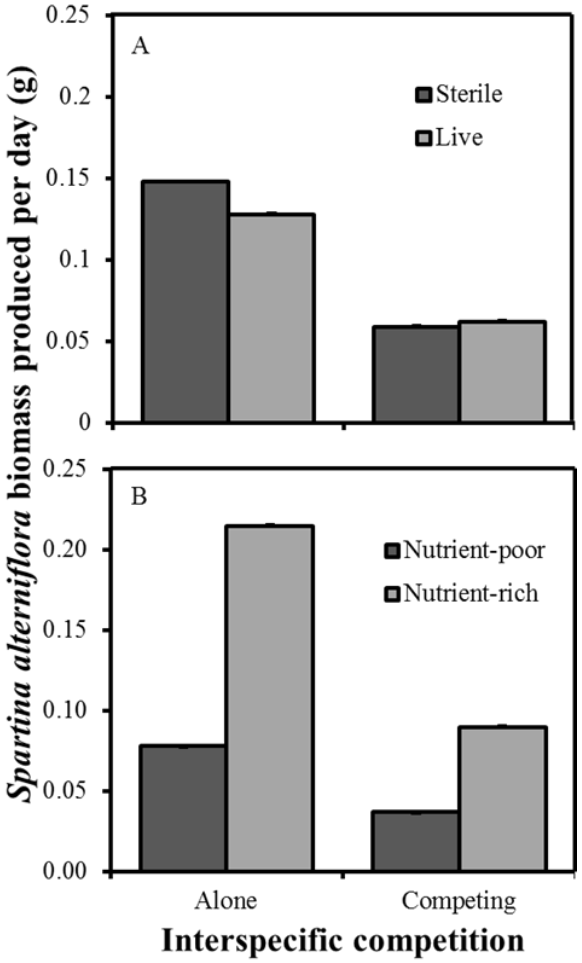

Figure 4.2. Mean (± S.E.) biomass produced per growing day (g) for *Spartina alterniflora* grown alone or in competition with *Phragmites australis* in A) live or sterilized soil inoculum and B) nutrient-rich or nutrient-poor soil. Error bars are obscured due to their small size.

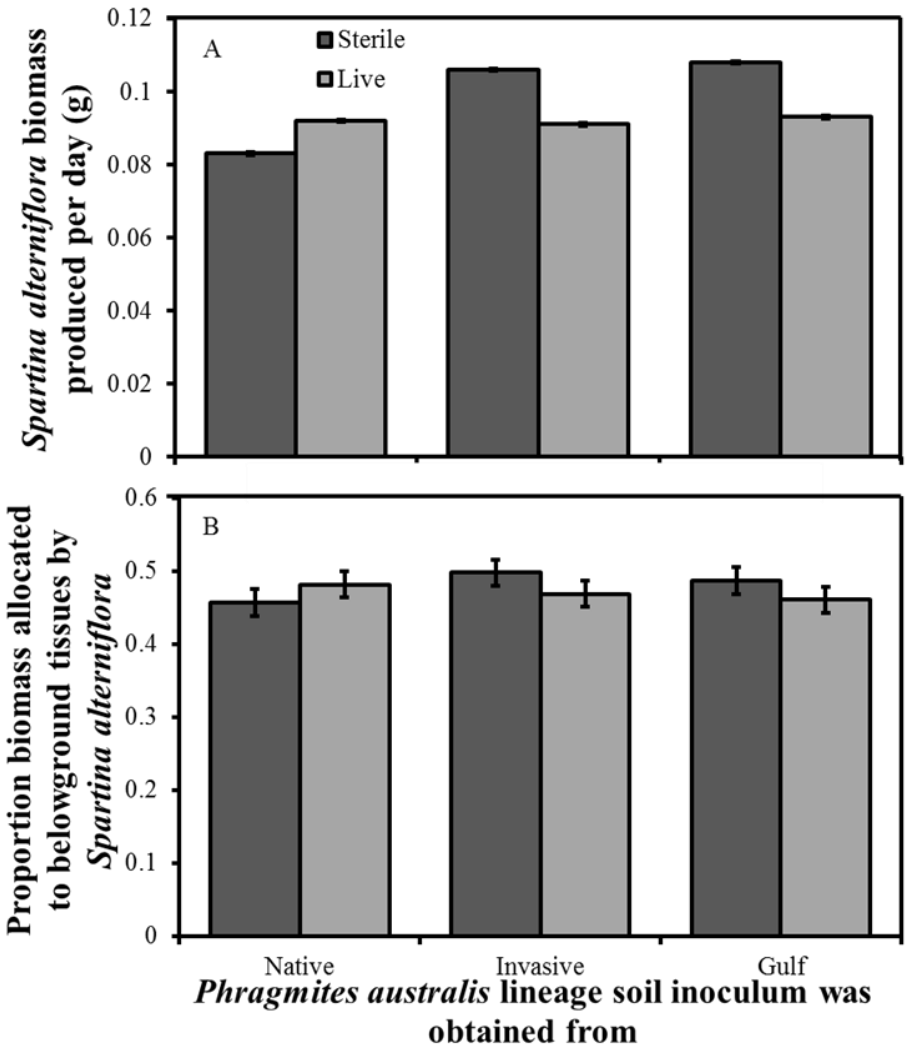

Figure 4.3. Impact of live or sterilized soil biota inoculum obtained from the three *Phragmites australis* lineages on A) mean (± S.E.) biomass produced per growing day (g) and B) mean (± S.E.) proportion of biomass allocated to belowground tissue (roots and rhizomes) for *Spartina alterniflora*. Error bars are obscured due to their small size.

Proportional biomass allocation to belowground tissues

Using AICc criteria, variation in the proportion of *P. australis* biomass allocated to belowground tissues was best explained by four models (Appendix C). The top model (AICc = -1222.0, AICc weight = 0.436) had at least 2.2 times the support of the other three models (second top model: AICc = -1220.4, ΔAICc = 1.63, AICc weight = 0.193, see Appendix C for additional details), and included *P. australis* lineage, live/sterile soil inoculum, presence/absence

of an interspecific competitor, high/low nutrient availability, and the lineage × nutrient availability interaction as influential explanatory variables. These variables were included in all four supported models (ΔAICc $\leq$ 2, cumulative AICc weight = 1), with the exception of live/sterile soil inoculum which was in three of the four models (cumulative AICc weight = 0.807), suggesting strong overall support for inclusion of these explanatory variables. For proportional biomass allocation to belowground tissues of *S. alterniflora*, the top model (AICc = -1153.6, AICc weight = 0.329) included *P. australis* lineage, live/sterile soil inoculum, presence/absence of an interspecific competitor, high/low nutrient availability as well as the lineage × soil inoculum and interspecific competitor × nutrient availability interactions (Appendix C). Again, all five plausible models included these same variables (cumulative AICc weight = 1), except for the competitor × nutrient availability interaction, which was in three of the models (cumulative AICc weight = 0.701).

P. australis exhibited a small (3%) increase in average proportional biomass allocation to belowground tissues in live (0.545 ± 0.039) versus sterile (0.531 ± 0.039) soil inoculum. For *S. alterniflora*, the effect of soil inoculum again depended upon the *P. australis* lineage the soil inoculum was sourced from (i.e., lineage × soil inoculum interaction). Proportional biomass allocation to belowground tissues increased by 3% in live soil inoculum compared to sterile soil inoculum for the native *P. australis* lineage, but decreased by 3% in pots containing live soil inoculum for the invasive lineages (Fig. 4.3B).

Proportional biomass allocation to belowground tissues of *P. australis* decreased 4% when plants were grown in competition with *S. alterniflora* (0.528 ± 0.039) than when grown alone (0.548 ± 0.039). In contrast, competition with *P. australis* led to increased proportional biomass allocation to belowground tissues of *S. alterniflora*, but the strength of this effect varied

with nutrient availability (i.e., competitor × nutrient availability interaction); 13% and 9% increases in nutrient-poor and nutrient-rich pots, respectively (Fig. 4.4). Moreover, when grown alone, *S. alterniflora* decreased proportional biomass allocation to belowground tissues by 21% in nutrient-rich versus nutrient-poor pots, compared to a 24% decrease when grown in competition with *P. australis* (Fig. 4.4).

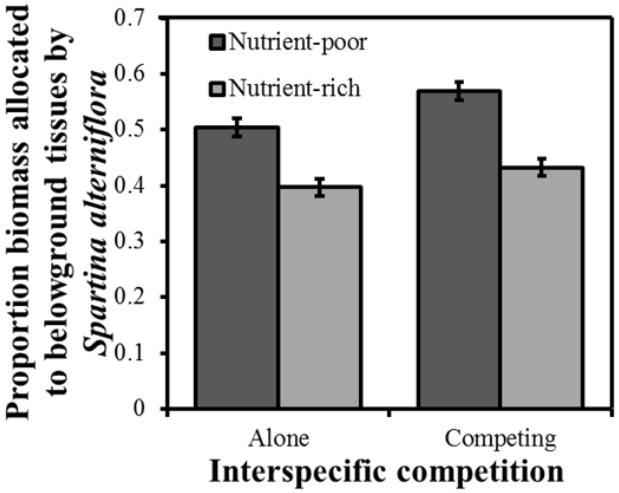

Figure 4.4. Mean (± S.E.) proportion of biomass allocated to belowground tissues (roots and rhizomes) for *Spartina alterniflora* grown alone or in competition with *Phragmites australis* and in nutrient-rich or nutrient-poor soil.

Differences among *P. australis* lineages in the proportional biomass allocation to belowground tissues were dependent upon nutrient availability (lineage × nutrient interaction). In nutrient-poor pots, the European lineage had the greatest proportional biomass allocation to belowground tissues, 10% and 34% higher than the native and Gulf lineages, respectively (Fig. 4.5). However, the ranking in proportional biomass allocation to belowground tissues for the native and European lineage reversed order in nutrient-rich pots. In this case, the proportional biomass allocation to belowground tissues was highest for the native lineage, 5% and 37% higher than the European and Gulf lineages, respectively. In comparison to the low nutrient

treatment, nutrient addition resulted in 18%, 32% and 16% lower proportional biomass allocated to belowground tissues for the native, European, and Gulf lineages, respectively (Fig 4.5).

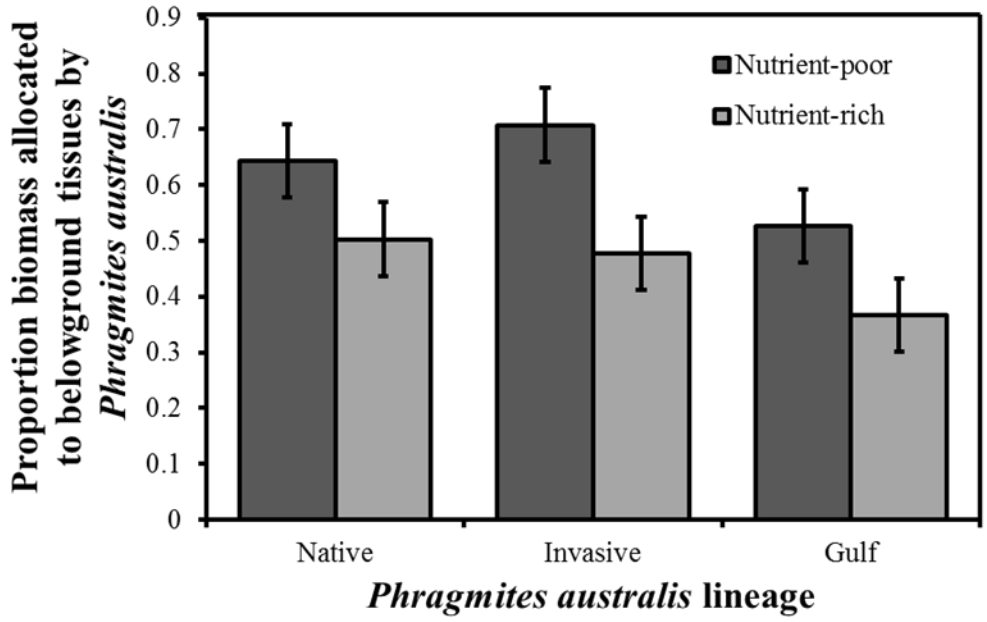

Figure 4.5. Mean (± S.E.) proportion of biomass allocated to belowground tissues (roots and rhizomes) for the three lineages of *Phragmites australis* grown in nutrient-rich and nutrient-poor soil.

DISCUSSION

Although it is clear that the interactions between plants and soil biota can play a critical role in plant invasions (Klironomos 2002; Agrawal et al. 2005; Kulmatiski et al. 2008; Suding et al. 2013), their indirect effects, context dependency, and relationships with other processes linked to species invasions are only just beginning to be explored (e.g., Larios and Suding 2015). In the first study to examine the net impact of soil biota on *P. australis,* we found that live soil biota reduced daily biomass production by 11% and increased proportional biomass allocation to belowground tissues by 3%, irrespective of lineage, presence of an interspecific competitor, or nutrient availability. Thus, harmful soil biota appears to consistently dominate PSFs involving *P. australis*. Coupled with the lack of variation in PSFs among *P. australis* lineages, this consistent

negative impact strongly suggests that interactions with soil biota do not directly facilitate the relative success of invasive *P. australis* in North America. In contrast, soil biota collected from the rhizosphere of invasive *P. australis* populations (European and Gulf lineages) caused a reduction in daily biomass production and an increase in proportional biomass allocation to belowground tissues of native *S. alterniflora*, whereas soil biota from native *P. australis* populations had the opposite effect. Interestingly, regardless of lineage, PSFs involving *P. australis* soil biota were negative for *S. alterniflora* grown alone but positive when grown in the presence of *P. australis*, suggesting that harmful generalist soil biota prefer *P. australis* but will attack *S. alterniflora* if it is the only available host. To our knowledge, this is the first study to demonstrate that the direction of soil legacies can change depending upon the presence/absence of the invasive plant, and also differ among closely-related native and invasive taxa. Our study also supported *P. australis* as a dominant competitor of native marsh plants, but provided little evidence that the invasive lineages have superior competitive ability compared to the native lineage, suggesting that interspecific competition may not be an important factor driving *P. australis* invasion in southern wetlands. However, the European invasive lineage had a stronger plastic response in biomass allocation than the native and Gulf lineages which may result in a competitive advantage with different environmental conditions or longer-term experiments. Moreover, *P. australis* and *S. alterniflora* differed in plasticity of biomass allocation in response to soil biota and interspecific competition, suggesting they may each be limited by different resources (i.e., light versus nutrients) in each of these interactions. The direct and indirect effects of soil biota, interspecific competition, and soil biota identified in this study can have important implications for understanding invasion success and impacts of *P. australis*, and for the restoration of invaded areas.

Prediction 1: Invasive *P. australis* lineages experience more positive PSFs than the native lineage.

Many invasive plants benefit from more positive plant-soil feedbacks relative to co-occurring native species (e.g., Klironomos 2002; Agrawal et al. 2005; Van Grunsven et al. 2007; Kulmatiski et al. 2008; MacDougall et al. 2011; Suding et al. 2013), generally attributed to enemy release from harmful biota present in the native range (e.g., Beckstead and Parker 2003; Reinhart et al. 2003) or beneficial associations with native or co-introduced mutualists in the introduced range (Richardson et al. 2000b; Rodríguez-Echeverría 2010). In our study, the presence of live soil biota derived from the rhizosphere of each *P. australis* population resulted in an 11% average decrease in daily *P. australis* biomass production and a 3% increase in proportional biomass allocation to belowground tissues. Because we examined net impact, these effects represent relative dominance of damaging soil-borne microbial pathogens, parasites, and herbivores over beneficial interactions with mutualists. It is also important to note that all of our estimates of soil biota effects could be considered conservative due to the use of a soil inoculum ratio of just 10% of total soil weight (Brinkman et al. 2010). PSFs may also increase in magnitude over time (e.g., Diez et al. 2010; Hawkes et al. 2013), meaning that effects may have been even stronger if examined over more than one growing season.

Despite strong differentiation of rhizosphere microbe communities among *P. australis* lineages (Nelson and Karp 2013; Yarwood et al. in press; Bowen et al. in review) and the variable impact of some commonly isolated pathogens (*Pythium* spp.) on native and European *P. australis* seedlings (Crocker et al. 2015), we found that the negative impact of soil biota was consistent for all three lineages. Thus, in contrast to our first prediction, the invasive *P. australis* lineages do not benefit from a more positive PSF than the native lineage. This unexpected result

suggests that soil microbes do not directly impact invasion success of the European and Gulf *P. australis* lineages in North America. A possible reason for the lack of differences in PSF strength among lineages could simply be that although lineages differ in their microbial communities, their net effects on the plant are the same (i.e., despite high taxonomic turnover, functional turnover may be limited). However, this explanation is contradicted by Wagg et al. (2015) who demonstrated that differences in PSFs of two populations of *Trifolium pratense* could largely be explained by corresponding differences in the rhizosphere microbe community. In the only other study of intraspecific variation in PSFs that we are aware of, Bukowski and Petermann (2014) also identified strong variation in PSFs among accessions of *Arabidopsis thaliana*. Alternatively, *P. australis* is host to a diverse and potentially damaging oomycete pathogen community in some locations in Europe (Nechwatal et al. 2008). It is probable that this soil community was introduced alongside the European *P. australis* lineage, or has recently arrived (i.e., pathogen accumulation; Flory and Clay 2013), meaning that European *P. australis* in North America may experience similar negative feedbacks to in their native range. Therefore, a logical next step to investigating the influence of soil biota on *P. australis* invasion would be to compare PSFs between the native and introduced ranges.

The presence of live soil biota increased *P. australis* proportional biomass allocation to belowground tissues by 3%, possibly as a response to escape from soil-borne pathogens by growing away from the site of infection (e.g., D'Hertefeldt and van der Putten 1998) or to improve nutrients and water acquisition which may be compromised by harmful soil biota. Like daily biomass production, this effect was also independent of *P. australis* lineage. These results are consistent with previous studies which also demonstrated that soil biota can alter biomass

allocation patterns of invasive plants (Streitwolf-Engel et al. 1997; D'Hertefeldt and van der Putten 1998; te Beest et al. 2009; Gao et al. 2013).

Prediction 2: Spillover of soil biota from invasive lineages has more negative effects on *S. alterniflora* than soil biota from the native lineage

Our study suggests that generalist soil biota associated with *P. australis* also influence co-occurring native plants such as *S. alterniflora*. Importantly, soil biota from the rhizosphere of populations of the two invasive lineages had a net negative impact on *S. alterniflora* daily biomass production and proportional biomass allocation to belowground tissues, whereas soil biota from populations of the native lineage had a net positive impact on these variables. One possible explanation for the negative impact on *S. alterniflora* could be that invasive *P. australis* lineages suppress mutualisms between native plant species and beneficial soil biota (e.g., Stinson et al. 2006; Jordan et al. 2012), shifting the balance in favor of harmful soil biota. However, this explanation is contradicted by the positive impact of soil biota observed when *P. australis* was present as a competitor (see below). Alternatively, invasive *P. australis* may accumulate local generalist pathogens, which spillover onto *S. alterniflora*, dominating any positive impacts from beneficial organisms (e.g., Niu et al. 2007; Mangla et al. 2008). Interestingly, Li et al. (2014) previously demonstrated this phenomenon occurring between *P. australis* and *S. alterniflora* in the Dongtan wetland of the Chinese Yangtze River estuary, but the roles of the species were reversed; *S. alterniflora* is invasive in China and spillover of the fungal pathogen *Fusarium palustre* resulted in significant dieback of native *P. australis*.

Existing theory suggests that, given the negative PSFs for *P. australis* in this system, coexistence may be possible between *S. alterniflora* and *P. australis* (Bever et al. 1997; Reynolds et al. 2003) because negative PSFs promote coexistence through altering competitive

interactions and inducing competitive oscillations (Bever et al. 1997; Bever 2003; Reynolds et al. 2003; Revilla et al. 2013). However, our results suggest that spillover of beneficial soil biota represents an additional mechanism explaining why the native *P. australis* lineage generally co-occurs with a diverse range of other native species (Meyerson et al. 2009). In contrast, native plants may be excluded by spillover of pathogens and/or other harmful soil biota from the European and Gulf lineages. Suppression of the native plant community may assist the invasive *P. australis* lineages in forming extensive monocultures (Meyerson et al. 2000) because native plants decrease colonization success of *P. australis* seedlings (Minchinton and Bertness 2003) and reduce sprouting from rhizomes (Wang et al. 2006; Peter and Burdick 2010). Finally, because native *P. australis* commonly occurs in a mixed plant community, the soil collected from these populations may inherently contain more generalist soil biota also coadapted to interact with other native species.

Interestingly, *S. alterniflora* increased allocation by 3% when PSFs were positive and decreased proportional biomass allocation to belowground tissues by 3% when PSFs were negative – the opposite to *P. australis*. These findings suggest that both *S. alterniflora* and *P. australis* respond plastically to PSFs by altering proportional biomass allocation to belowground tissues, but with opposing strategies. These different responses could have potential long-term consequences for competition unable to be detected over a single growing season. For example, by increasing allocation to belowground biomass in response to negative PSFs, *P. australis* could actually gain a competitive advantage if nutrients are limiting. However, along with escaping harmful soil biota, *S. alterniflora* may indirectly benefit from their response to negative PSFs through increased plant height, specific leaf area, and photosynthetic capacity (Pattison et al. 1998; DeWalt et al. 2004; Meyer and Hull-Sanders 2008), particularly if light is a limiting factor.

In contrast, when PSFs are positive, *S. alterniflora* may benefit by investing more in belowground tissue to increase the frequency and strength of interactions with beneficial soil biota. Given the relative heights of *P. australis* (up to 5 m) and *S. alterniflora* (up to 1.5 m) (W. J. Allen and J. T. Cronin, pers. obs.), these contrasting strategies appear practical.

Prediction 3): The direction and strength of PSFs and spillover depends on the presence of an interspecific competitor and nutrient availability

The impacts of soil biota on *P. australis* daily biomass production and proportional biomass allocation to belowground tissues were unaffected by the presence of *S. alterniflora* as a competitor or availability of nutrients, suggesting there is little context dependency of *P. australis* PSFs in regards to these variables. However, in support of our third prediction, live soil biota decreased daily biomass production of *S. alterniflora* by 14% when grown alone, but increased daily biomass production by 5% when competing with *P. australis*. This interesting finding could be explained by a couple of different scenarios: First, harmful generalist soil biota may prefer to interact with *P. australis* over *S. alterniflora* and only switch host when *P. australis* is absent. Such a preference is not entirely unexpected given that the soil inoculum was originally collected from natural *P. australis* populations and thus probably includes organisms coadapted to that particular lineage and population (Bowen et al. in review). Therefore, we suggest that *P. australis* generates a negative soil legacy whereby generalist soil biota switch to native host species when *P. australis* is unavailable. Negative soil legacies appear to be relatively common among invasive species and are widely-recognized to prevent establishment of native plants and improve chances of recolonization by invasives (e.g., Eppinga et al. 2006; Mangla et al. 2008; Grman and Suding 2010; Rodríguez-Echeverría et al. 2013; Grove et al. 2015). Second, our findings could be indicative of spillover of beneficial soil biota from *P. australis* to *S.*

alterniflora (i.e., an apparent mutualism), suggesting that *P. australis* may indirectly facilitate the growth of co-occurring native plants. Moreover, our findings also indicate that this apparent mutualism may be more likely with populations of native lineage. Due to the nature of examining net impacts of soil biota, these two mechanisms cannot easily be disentangled without identifying the organisms involved, which was outside the scope of this study.

Prediction 4: Invasive lineages of *P. australis* possess stronger interspecific competitive ability than native lineages and *S. alterniflora*

Interspecific competition is an important factor in structuring plant communities (Grime 1973; Tilman 1982) and superior competitive ability has long been recognized as a common trait of invasive plant species (Elton 1958; Vilà and Weiner 2004; Gioria and Osborne 2014). In this study, we found that the presence of a competitor decreased biomass production of *P. australis* and *S. alterniflora* by 13% and 57%, respectively. In support of our fourth prediction, the more than four-fold higher impact of interspecific competition on *S. alterniflora* than *P. australis* clearly identifies *P. australis* as the superior competitor. This result is consistent with studies showing that *S. alterniflora* tends to be restricted to lower marsh areas due to its superior tolerance of abiotic stress factors such as high salinity and flooding but relatively poor competitive ability (Bertness 1991; Pennings et al. 2005).

Superior competitive ability is commonly cited as one of the main reasons the European *P. australis* lineage has become so prevalent in North America (e.g., Howard et al. 2008, Holdredge et al. 2010) and a number of studies have indicated that European *P. australis* is a stronger competitor than the native and Gulf lineages (Saltonstall and Stevenson 2007; Howard et al. 2008; Holdredge et al. 2010; Chow 2014). In contrast to these studies and our fourth prediction, we failed to find any differences in interspecific competitive ability among the three

P. australis lineages or their competitive impact on *S. alterniflora*. Interestingly, Chow (2014) found that competitive ability of native and invasive lineages may be more similar at lower latitudes in North America. Because the *P. australis* populations used in our experiment were from these low latitudes, this pattern may explain the lack of observed differences in competitive ability among lineages. Thus, we suggest that interspecific competitive ability may not be a key factor explaining the predominance of European relative to native and Gulf *P. australis* in North America, particularly at low latitudes.

Interspecific competition also prompted plastic changes in biomass allocation of both *P. australis* and *S. alterniflora*. Similar to the results of soil biota, these changes were in opposite directions. *P. australis* increased biomass allocation to aboveground tissues by 4% when competing with *S. alterniflora*, whereas *S. alterniflora* increased biomass allocation to belowground tissues by 11%. Shifts in biomass allocation in response to competition are varied and likely depend upon a number of factors including whether belowground or aboveground resources are more limiting (Poorter et al. 2011). Thus, our findings suggest that when competing, *P. australis* is limited by light and *S. alterniflora* by nutrient or water availability. Alternatively, increasing belowground storage could represent a strategy to store existing resources in rhizomes until growth conditions are improved (e.g., Cheplick and Gutierrez 2000).

Prediction 5: PSFs and nutrient availability alter interspecific competition between *P. australis* and *S. alterniflora*

Soil biota can play a significant role in altering outcomes of interspecific competition (e.g., Marler et al. 1999; Casper and Castelli 2007; Hodge and Fitter 2013). In our study, live soil biota and nutrient availability did not affect the outcome of interspecific competition for *P. australis*. However, live soil biota reduced the impact of interspecific competition on *S.*

alterniflora daily biomass production to 52% in comparison to 60% with sterile soil biota, supporting our fifth prediction. This result can likely be attributed to the negative PSF suffered by *P. australis* which may decrease its competitive ability or the strength of the apparent mutualism (i.e., spillover) affecting *S. alterniflora*. This result provides further support for the possibility of coexistence between *P. australis* and *S. alterniflora*, and suggests that it could be mediated by PSFs.

In further support of our prediction, the effects of interspecific competition on daily biomass production and proportional biomass allocation to belowground tissues of *S. alterniflora* also depended on nutrient availability. In nutrient rich pots, the impacts of interspecific competition on *S. alterniflora* biomass production and allocation to belowground tissues were increased by 6% and 4%, respectively, relative to nutrient-poor pots. This is in contrast to studies by Levine et al. (1998) and Emery et al. (2001) who demonstrated that nutrient addition decreased negative impacts of interspecific competition on *S. alterniflora* (i.e., it became a dominant competitor in nutrient-rich environments). However, their experiments did not include *P. australis*, which has one of the highest nitrogen use efficiencies of all land plants (Mozdzer et al. 2013). Finally, Medeiros et al. (2013) demonstrated that the competitive ability of *S. alterniflora* relative to *P. australis* increases with salinity. Thus, the outcome of interspecific competition between these two species may change under varying environmental conditions not tested in this study.

Prediction 6: Invasive *P. australis* lineages respond more positively to increased nutrient availability than the native lineage and *S. alterniflora*

Unsurprisingly, nutrient availability had a strong influence on all dependent variables in our study. Of particular interest, the influence of nutrient availability on *P. australis* biomass

allocation varied among lineages, supporting our sixth prediction. The decrease in proportional biomass allocation to belowground tissues in response to added nutrients was nearly two times greater for the European lineage than the native and Gulf lineages, suggesting that the European lineage exhibits greater phenotypic plasticity in biomass allocation. Previous studies have shown that invasive species, including European invasive *P. australis* (Chapter 3; Bhattarai et al. in review), regularly benefit from greater phenotypic plasticity relative to closely-related native species, particularly in response to nutrient availability (Richards et al. 2006; Davidson et al. 2011). Indeed, increased nutrient deposition via disturbance and anthropogenic modification is considered to be a major contributing factor to *P. australis* invasion success (Bertness et al. 2002; Silliman and Bertness 2004; Holdredge et al. 2010). Along with the strong plastic shifts in biomass allocation observed in this study, European invasive *P. australis* also enjoys higher maximum nutrient uptake ability than the native lineage (Mozdzer et al. 2010) and can alter its nitrogen metabolism to match conditions (Mozdzer and Megonigal 2012). Thus, although the differences in biomass allocation among lineages did not translate to differences in daily biomass production, our results suggest that over a longer time period (i.e., more than one growing season) the European invasive lineage may achieve a competitive advantage through its stronger plastic response to nutrient availability.

Prediction 7: Plant responses to nutrient availability are influenced by soil biota and interspecific competition

The influence of nutrient availability on *P. australis* was unaffected by the presence of live soil biota and interspecific competitors, suggesting that the harmful effects of negative PSFs and interspecific competition do not impact nutrient uptake efficiency of *P. australis* or its strong plastic response to nutrient availability. However, in partial support of our seventh prediction,

competition with *P. australis* reduced the ability of *S. alterniflora* to benefit from increased nutrient availability. The proportional increase in daily biomass production of *S. alterniflora* due to increased nutrients was lower when competing, in contrast to the stronger decrease in proportional biomass allocation to belowground tissues. This means that when competing with *P.* australis, *S. alterniflora* opted for higher allocation to aboveground tissues despite the high nutrient availability, likely due to a shift in the balance of limiting resources due to shading from the taller *P. australis.*

Conclusions and implications for restoration

The importance of soil biota to many aspects of plant ecology is well established. Contrary to expectations, our study suggests that interactions with soil biota do not directly influence the success of invasive *P. australis* lineages but instead have more subtle, indirect impacts in this system. Specifically, we establish that soil biota associated with *P. australis* can impact native plant species via altered interspecific competition strength, spillover of pathogens or mutualists (i.e., apparent competition and mutualism), and soil legacy effects even once the original host plant has been removed. These indirect effects have the potential to promote coexistence of native plants in populations of the native *P. australis* lineage and exclusion in invasive *P. australis* populations (Bever et al. 1997; Reynolds et al. 2003). Consistent with other studies, we also found *P. australis* to be a dominant interspecific competitor and to possess a strong plastic response to nutrient availability. However, we found little support for the hypothesis that the invasive lineages have superior competitive ability compared to the native lineage within a single growing season.

From a restoration perspective, the identity and impact of the soil community should be a crucial consideration when attempting to restore habitat occupied by invasive plant species

(Eviner and Hawkes 2008; Corbin and D'Antonio 2012). Thus, we suggest that microbial inoculation (Middleton and Bever 2012), topsoil removal (e.g., Hölzel and Otte 2003), or planting the native *P. australis* lineage are potentially useful approaches to ameliorate the effects of harmful soil biota and promote cultivation of beneficial soil biota, with the goal of facilitating development of a diverse native community in areas where invasive *P. australis* is being managed. Successful restoration may be crucial to preventing re-establishment of invasive *P. australis* by providing greater resistance to colonization by seedlings and vegetative spread (Minchinton and Bertness 2003; Wang et al. 2006; Peter and Burdick 2010; Byun et al. 2013). To date, the use of native *P. australis* in restoration efforts has not been documented, so its effectiveness as a nursery species is unknown. Future studies should focus on the identification of lineage-specific pathogens or beneficial organisms which may be useful in novel management efforts focused on control of the invasive *P. australis* lineages or conservation/restoration of the native lineage (Kowalski et al. 2015). Finally, because invasive species interact directly and indirectly with a complex community of organisms and abiotic conditions, expanding PSF studies to multitrophic and community-level interactions, and continuing to address context dependency, is critical to furthering our understanding of the role of PSFs in plant invasions.

CHAPTER 5
CONCLUSIONS

In my dissertation, I examined the role of plant genetics and plasticity, biogeography, and multitrophic species interactions in driving plant invasions at large spatial scales. Specifically, I used a combination of field surveys along with greenhouse and common garden experiments to I focus on comparisons between native and invasive lineages of the cosmopolitan wetland grass *Phragmites australis*. My dissertation underscores the importance of placing biological invasions into a community context and taking a biogeographic approach to understanding their causes and impacts.

First, in Chapter 2, I demonstrated the importance of examining the role of species interactions in invasion success using more than just a pairwise species framework. I found evidence suggesting that an invasional meltdown may be underway in North America involving *P. australis* and a genus of co-introduced specialist herbivores, the *Lipara* gall-flies. This invasional meltdown appears to be mediated by classical enemy release of *Lipara* from arthropod predators and parasitoids in the native range of Europe, resulting in higher densities of *Lipara* in North America. In the introduced range, the native *P. australis* lineage suffers disproportionately higher herbivory than the invasive lineage (i.e., local enemy release for the invasive lineage), attributed to a combination of higher *Lipara* performance and four times less vertebrate predation on the native than invasive lineage. Moreover, recent evidence also suggests that apparent competition likely contributes to the higher herbivory observed on the native lineage (Bhattarai 2015), further supporting the invasional meltdown hypothesis. However, the role of these interactions is currently restricted to the distribution of *Lipara* on the east coast from North Carolina to Maine and sporadic reports from Michigan and Utah (Blossey 2014). This work illustrates the complex interactions that form when multiple interacting species are introduced

into a novel environment, and highlights the importance of applying a multitrophic framework to the study of biological invasions.

Such trophic interactions involving multiple introduced species are only likely to become more commonplace as invasive species become more prevalent and interact more frequently. Thus, a broader community-level perspective is becoming increasingly important as more introduced species spanning a range of trophic levels integrate into complex interaction networks (e.g., food webs) in novel native-invasive systems. Recent advances have provided a framework with which to begin investigating the general properties of species interaction networks which make them susceptible or resistant to invasion (Bartomeus et al. 2016; Hui et al. 2016). By quantifying trait-mediated interaction networks within this framework, invasion biologists should be able to move away from assessing "invasiveness" of a certain species or "invasibility" of a particular ecosystem in isolation from one another. Comparing network properties of uninvaded, invaded, and restored ecosystems would also enable a community-level approach to examining the impacts of invasive species (e.g., Albrecht et al. 2014) and success of restoration (e.g., Forup et al. 2008). The results of my dissertation suggest that future studies should incorporate multitrophic above and belowground interactions as well as consider biogeographic variation and context dependency based on local environmental conditions.

In Chapter 3, I investigated the relative role of local adaptation and phenotypic plasticity in driving latitudinal gradients in the strength of local enemy release of invasive *P. australis* from herbivory by *L. rufitarsis*, the most widespread and abundant *Lipara* species (Chapter 2). I discovered that local enemy release of the invasive *P. australis* lineage from *L. rufitarsis* in the field was a plastic response, driven through modification of stem characteristics (height, diameter, density) by local environmental conditions, rather than genetic differences between

native and invasive lineages. Furthermore, comparatively stronger local enemy release at northern than southern latitudes was generated by local adaptation of the native but not invasive lineage along a latitudinal gradient (i.e., non-parallel latitudinal gradients). This study adds to the growing body of evidence suggesting that non-parallel latitudinal gradients in herbivory between native and invasive taxa may be a common phenomenon which could have important implication for the establishment and/or spread of invasive plant species (Cronin et al. 2016; Bhattarai et al. in review). Moreover, my study contributes to our understanding of the evolutionary and environmental mechanisms responsible for these gradients and how they vary between native and invasive plant taxa.

To date, latitudinal gradients in species interactions of co-occurring native and invasive taxa have only been explored within one study system (*P. australis*) and a single type of interaction (herbivory) (Chapter 2, Cronin et al. 2015; Bhattarai et al. in review). Based on the evidence from these studies, it seems likely that non-parallel latitudinal gradients between native and invasive taxa are a common occurrence and potentially involve a diverse suite of organisms and types of species interactions. Therefore, future studies should focus on testing this hypothesis for other interactions influential to invasion success such as competition, predation/parasitism, plant-soil feedbacks, and mutualisms, both with *P. australis* and other model systems. Furthermore, this biogeographic approach could also be applied to the study of multitrophic interactions and ecological networks (see above). Research of this nature could lead to transformative insights into the relative contribution of local- and global-scale processes to the structure and function of communities, their resistance/susceptibility to invasion, and the impacts and management of invasive species. Another potentially fruitful approach may be to synthesize the extensive primary literature by conducting reviews and meta-analyses. For example, the

results of Chapter 3 and similar studies (Cronin et al. 2015; Bhattarai et al. in review) demonstrate that biotic resistance/susceptibility is likely to be stronger at more extreme latitudes (see Fig. 1.1 and 3.1 for examples). Thus, a meta-analysis testing the strength of local enemy release and/or biotic resistance against the geographic location of the study may address if this is a general biogeographic pattern.

In Chapter 4, I conducted the first reported study of plant-soil feedbacks involving *P. australis* using a fully crossed multi-factor greenhouse experiment which simultaneously assessed the effects of the presence/absence of an interspecific competitor (native smooth cordgrass, *Spartina alterniflora*) and nutrient-poor versus nutrient-rich environments. Soil biota from field populations reduced daily biomass production by 11% for all three *P. australis* lineages, suggesting that interactions with soil biota do not directly influence the success of invasive *P. australis* lineages in North America. Moreover, although competition and nutrient availability significantly impacted all variables, we also found little evidence supporting their role in invasion success of *P. australis*. However, one novel and significant result was that the effects of soil biota on *S. alterniflora* were variable; soil biota from invasive *P. australis* negatively affected *S. alterniflora*, whereas soil biota from native *P. australis* had a positive impact on *S. alterniflora*. To my knowledge, this is the first study to demonstrate that the direction of soil legacies can differ among closely-related native and invasive taxa. These results are particularly important from a restoration perspective because they highlight the need for consideration of soil legacies and pathogen/mutualist spillover when attempting to restore invaded habitats.

Despite advancing our understanding of which biotic and abiotic factors may explain invasion success of *P. australis*, the actual influence of these factors on plant fitness, spread and

impact remains to be quantified. For example, Cronin et al. (2015) found that damage from leaf chewing herbivores was 6.5 times higher on the native than invasive lineage. However, the percent of leaf area lost to chewing damage was only 0.013%, suggesting little to no effect on *P. australis* fitness, especially because all lineages exhibit tolerance of herbivory (Croy et al. in prep.). In comparison to the 11% decrease in biomass production caused by soil biota (Chapter 4), the effects of herbivores may be relatively insignificant. Thus, future studies should focus on quantifying demographic impacts of herbivores, which may help identify the most damaging species contributing to the loss of native *P. australis* populations. Moreover, although my dissertation demonstrates strong effects of soil biota on growth and biomass allocation of *P. australis* and its native neighbors, these estimates could be considered conservative due to the use of a soil inoculum ratio of just 10% of total soil weight (Brinkman et al. 2010). Plant-soil feedbacks may also vary temporally (e.g., Diez et al. 2010; Flory and Clay 2013; Hawkes et al. 2013), hence examining plant-soil feedbacks in natural conditions and over longer than a single growing season should be a priority for future studies.

Finally, my dissertation research has significant implications for the management of habitat occupied by *P. australis*. Current management approaches (typically herbicide and physical removal) targeting control of the invasive lineage are costly (Martin and Blossey 2013), relatively ineffective (Hazelton et al. 2014), and can result in non-target mortality of the native lineage. A classical biological control program focusing on arthropod herbivores has also been underway for close to two decades (Schwarzländer and Häfliger 2000; Tewksbury et al. 2002; Häfliger et al. 2005, 2006; Blossey 2014). However, my dissertation has contributed to growing concern that the introduction of these species has the potential to be highly detrimental to the native *P. australis* lineage (Bhattarai et al. 2016; Cronin et al. 2016). This obstacle suggests that

an alternative approach is probably required if the goal is to concurrently control and conserve invasive and native populations, respectively. The urgent need to find a means to effectively manage *P. australis* has been highlighted by the formation of the Global Phragmites Network (an international collaborative research group of which I am a founding member) (Packer et al. in review) and the Great Lakes *Phragmites* Collective (http://greatlakesphragmites.net/), as well as the release of recent special issues dedicated to *P. australis* in the journals *AoB Plants* and *Biological Invasions*.

In light of the current lack of effective management approaches, a recent body of literature has begun exploring the possibility of applying novel microbial approaches to *P. australis* management (see Kowalski et al. 2015 for review). Such approaches could focus on developing specialized pathogenic microbes as biological control agents for the invasive *P. australis* lineage, disrupting positive interactions between invasive *P. australis* and soil/endophytic microbes, or promoting beneficial interactions involving the native lineage. Future studies should identify and further investigate the specificity and impact of pathogen species responsible for the consistent net negative impact of soil biota on *P. australis* (Chapter 4). Moreover, the differing impacts on native plants of soil legacies and pathogen spillover from invasive and native *P. australis* could be important to restoration practices. For example, soil remediation (e.g., microbial inoculation, planting native *P. australis*, topsoil removal) may be crucial to promoting a beneficial soil biota and facilitating the development of a diverse native community. Continuing to pursue these avenues of research will ultimately provide insight into how to best predict, prevent, and manage biological invasions.

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APPENDIX A. SUPPLEMENTARY MATERIAL FOR CHAPTER 2

PHRAGMITES AUSTRALIS POPULATIONS VISITED IN EUROPE

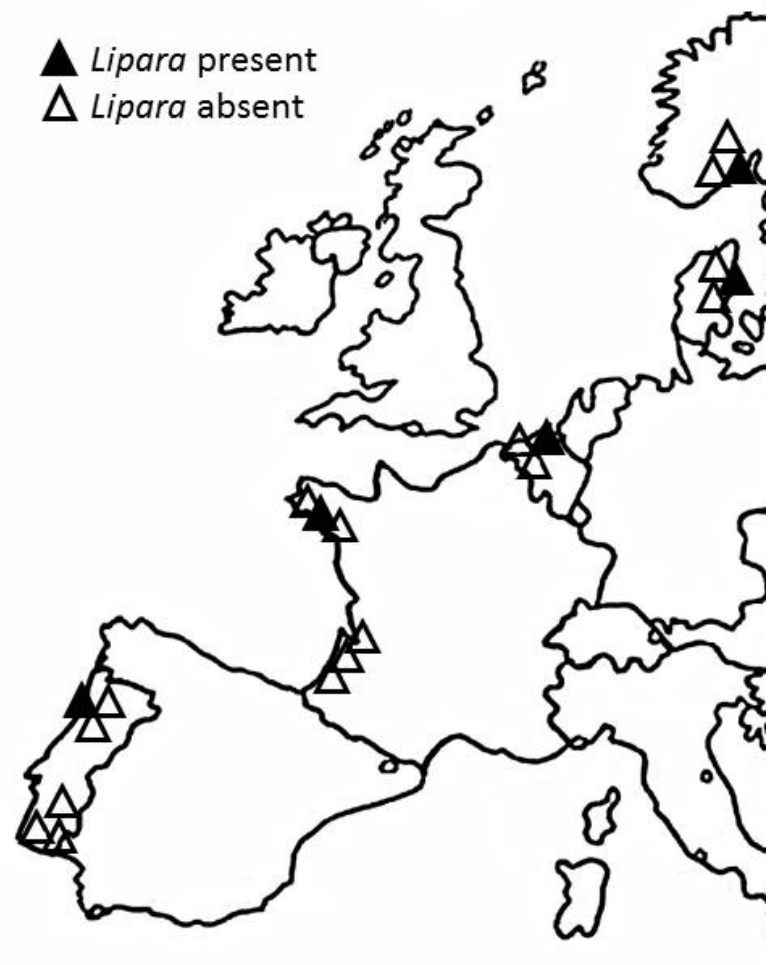

Figure A.1. Location of *Phragmites australis* sampling sites in Europe.

PHRAGMITES AUSTRALIS POPULATIONS VISITED IN NORTH AMERICA AND EUROPE

Table A.2. List of *Phragmites australis* patches surveyed for *Lipara* in North America and Europe, including site name, country/state/province, latitude, longitude, *P. australis* genotype (M/L1 = invasive, I = Gulf Coast, N = native), sampling period (summer 2012, winter 2013, summer 2013, summer 2014), and the total number of *Lipara* galls collected from each patch for species identification. An * indicates that galls were collected and dissected in summer 2012 and a † indicates galls were collected for rearing and dissection in winter 2013.

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
Arizona State University	Arizona	33.43	-111.93	N	Summer 2012	0
Tortilla Flats	Arizona	33.53	-111.39	N	Summer 2012	0
McHook Park	Arizona	34.97	-110.64	N	Summer 2012	0
Glen Canyon	Arizona	36.86	-111.60	N	Summer 2012	0
Little Rock	Arkansas	34.69	-92.29	M	Summer 2013	0
Greeson Wash	California	32.68	-115.61	I	Summer 2014	0
Calexico	California	32.69	-115.47	I	Summer 2014	0
Agua Caliente Hot Springs	California	32.95	-116.30	N	Summer 2013 + Summer 2014	0
Salt Creek	California	33.45	-115.84	I	Summer 2014	0
Torres Martinez Preserve	California	33.54	-116.10	N	Summer 2014	0
Thousand Palms Canyon	California	33.83	-116.31	N	Summer 2013	0
Santa Clara River	California	34.36	-119.01	N	Summer 2013	0
Bum Paradise	California	34.54	-117.29	N	Summer 2014	0
I-40 Border	California	34.72	-114.49	M	Summer 2013 + Summer 2014	0
Catfish Paradise	California	34.74	-114.49	N	Summer 2014	0
Zzxzyx Road	California	35.17	-116.11	N	Summer 2013	0
Salinas River	California	35.50	-120.65	M	Summer 2013 + Summer 2014	0
Jim Andre	California	36.21	-117.99	N	Summer 2014	0
Lubkin Canyon Rd	California	36.54	-118.07	N	Summer 2014	0
Bishop	California	37.36	-118.42	N	Summer 2014	0
Route 395	California	37.39	-118.50	N	Summer 2014	0
Lower Rock Creek	California	37.43	-118.56	N	Summer 2014	0

(Table A.2 continued)

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
McNabney Marsh	California	38.03	-112.11	M	Summer 2014	0
Pettipaug Invasive*	Connecticut	41.36	-72.38	M	Summer 2012	20
Pettipaug Invasive 2†	Connecticut	41.36	-72.38	M	Winter 2013	52
Pettipaug Native*†	Connecticut	41.36	-72.38	N	Summer 2012 + Winter 2013	269
Appoquinimink Invasive*	Delaware	39.45	-75.65	M	Summer 2012 + Winter 2013	35
Appoquinimink Invasive 2†	Delaware	39.45	-75.65	M	Winter 2013	80
Appoquinimink Native†	Delaware	39.45	-75.65	N	Winter 2013	38
John Prince	Florida	26.60	-80.08	I	Summer 2012	0
Okeeheelee	Florida	26.66	-80.17	I	Summer 2012	0
Okeeheelee 2	Florida	26.66	-80.17	I	Summer 2012	0
Granite City	Illinois	38.66	-90.09	M	Summer 2013	0
Sand Prairie	Illinois	38.67	-90.07	M	Summer 2013	0
Litchfield	Illinois	39.15	-89.67	M	Summer 2013	0
Exit 96	Illinois	39.80	-89.59	M	Summer 2013	0
Lincoln	Illinois	40.23	-89.27	M	Summer 2013	0
I-39 2	Illinois	40.88	-89.04	M	Summer 2013	0
Oglesby	Illinois	41.30	-89.08	M	Summer 2013	0
Mendota	Illinois	41.51	-89.05	M	Summer 2013	0
I-39 1	Illinois	41.97	-89.02	M	Summer 2013	0
Rosecrans	Illinois	42.46	-87.91	M	Summer 2013	0
Forney Lake	Iowa	40.85	-95.78	N	Summer 2013	0
Mondamin	Iowa	41.76	-96.03	N	Summer 2013	0
Mondamin 2	Iowa	41.78	-96.04	N	Summer 2013	0
Ruthven 1	Iowa	43.16	-94.89	N	Summer 2013	0
Ruthven 2	Iowa	43.16	-94.92	N	Summer 2013	0
Ruthven 3	Iowa	43.17	-94.88	N	Summer 2013	0
Rockefeller Road	Louisiana	29.69	-92.84	M	Summer 2012 + Summer 2014	0
Rockefeller Road Extra	Louisiana	29.71	-92.83	M	Summer 2014	0

(Table A.2 continued)

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
Rockefeller Boat Launch	Louisiana	29.72	-92.77	I	Summer 2012 + Summer 2013	0
East Cameron	Louisiana	29.78	-93.29	M	Summer 2012 + Summer 2013	0
Cameron Jetty	Louisiana	29.78	-93.34	M	Summer 2012 + Summer 2013	0
Intracoastal City	Louisiana	29.79	-92.20	I	Summer 2012 + Summer 2013	0
Creole 1	Louisiana	29.84	-93.11	I	Summer 2012 + Summer 2013	0
Creole 2	Louisiana	29.84	-93.08	M	Summer 2012	0
Lake Fausse Point	Louisiana	29.94	-91.55	I	Summer 2013	0
Bonnet Carre Spillway	Louisiana	30.06	-90.37	I	Summer 2012 + Summer 2014	0
Pontchartrain	Louisiana	30.30	-90.40	I	Summer 2014	0
Pontchartrain 2	Louisiana	30.34	-90.41	I	Summer 2014	0
Fontainbleau 1	Louisiana	30.34	-90.03	I	Summer 2013	0
Fontainbleau 2	Louisiana	30.34	-90.05	I	Summer 2013	0
Webhannett Invasive*†	Maine	43.30	-70.58	M	Summer 2012 + Winter 2013	181
Webhannett Native*†	Maine	43.30	-70.58	N	Summer 2012 + Winter 2013	126
Nonesuch Native*†	Maine	43.58	-70.33	N	Summer 2012 + Winter 2013	181
Sawyer Rd Invasive*	Maine	43.59	-70.26	M	Summer 2012	22
Spurlink Native*	Maine	43.59	-70.25	N	Summer 2012	86
Yarmouth Invasive*†	Maine	43.80	-70.17	M	Summer 2012 + Winter 2013	104
Sherman Marsh Invasive	Maine	44.02	-69.60	M	Winter 2013	0
Sherman Marsh Native	Maine	44.02	-69.60	N	Winter 2013	0
Choptank Invasive*†	Maryland	38.77	-75.97	M	Summer 2012 + Winter 2013	95
Choptank Native*†	Maryland	38.77	-75.97	N	Summer 2012 + Winter 2013	132
East Sandwich Invasive†	Massachusetts	41.74	-70.44	M	Winter 2013	28
East Sandwich Native†	Massachusetts	41.74	-70.44	N	Winter 2013	27
Pleasant Prairie	Minnesota	42.53	-87.95	M	Summer 2013	0
Sherburne	Minnesota	43.69	-94.73	N	Summer 2013	0
Mankato	Minnesota	44.24	-94.03	N	Summer 2013	0
St. Peter 1	Minnesota	44.32	-93.94	N	Summer 2013	0

(Table A.2 continued)

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
St. Peter 2	Minnesota	44.33	-93.92	N	Summer 2013	0
St. Peter 3	Minnesota	44.48	-93.92	N	Summer 2013	0
Black Dog 1	Minnesota	44.80	-93.28	N	Summer 2013	0
Black Dog 2	Minnesota	44.81	-93.25	N	Summer 2013	0
Centerville	Minnesota	45.17	-93.07	N	Summer 2013	0
Lino Lakes	Minnesota	45.19	-93.08	N	Summer 2013	0
I-35E 1	Minnesota	45.22	-93.03	N	Summer 2013	0
Eureka	Minnesota	45.26	-93.02	N	Summer 2013	0
Forest Lake	Minnesota	45.27	-93.01	N	Summer 2013	0
Brook Park	Minnesota	45.91	-92.97	N	Summer 2013	0
MN10-1	Minnesota	46.42	-95.09	N	Summer 2013	0
Sebeka	Minnesota	46.62	-95.09	N	Summer 2013	0
Sky Harbor	Minnesota	46.74	-92.06	N	Summer 2013	0
Two Harbors	Minnesota	47.04	-91.77	N	Summer 2013	0
Cohasset	Minnesota	47.25	-93.59	N	Summer 2013	0
Aspen Ave 1	Minnesota	47.30	-93.71	N	Summer 2013	0
Ball Club	Minnesota	47.32	-93.95	N	Summer 2013	0
Aspen Ave 2	Minnesota	47.32	-93.25	N	Summer 2013	0
Laporte	Minnesota	47.36	-94.73	N	Summer 2013	0
MS Headwaters	Minnesota	47.36	-94.73	N	Summer 2013	0
Makinen	Minnesota	47.37	-92.32	N	Summer 2013	0
Aurora	Minnesota	47.37	-92.14	N	Summer 2013	0
Eveleth	Minnesota	47.37	-92.51	N	Summer 2013	0
Whipperwill	Minnesota	47.86	-89.92	N	Summer 2013	0
Missouri 7	Missouri	38.41	-90.34	M	Summer 2013	0
Telegraph	Missouri	38.42	-90.34	M	Summer 2013	0
Gravois Bluffs	Missouri	38.51	-90.43	M	Summer 2013	0
Squaw Creek	Missouri	40.06	-95.24	N	Summer 2013	0

(Table A.2 continued)

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
CampNB Native	New Brunswick	48.05	-66.66	N	Summer 2012	0
Baie de Chaleurs Invasive	New Brunswick	48.10	-66.30	M	Summer 2012	0
Estell Manor Invasive*†	New Jersey	39.41	-74.74	M	Summer 2012 + Winter 2013	143
Estell Manor Native*†	New Jersey	39.42	-74.73	N	Summer 2012 + Winter 2013	124
Southwest 1	New Mexico	31.80	-106.56	I	Summer 2012	0
Southwest 2	New Mexico	32.13	-106.68	I	Summer 2012	0
Las Cruces	New Mexico	32.52	-106.97	I	Summer 2012	0
Mackey Native Bridge*†	North Carolina	36.51	-75.95	N	Summer 2012 + Winter 2013	177
Mackey Invasive*	North Carolina	36.52	-75.95	M	Summer 2012	13
Mackey Invasive 2†	North Carolina	36.52	-75.96	M	Winter 2013	37
Mackey Native 2*†	North Carolina	36.52	-75.95	N	Summer 2012 + Winter 2013	170
Port Orford	Oregon	42.76	-124.50	N	Summer 2013	0
La Pocatiere Invasive	Quebec	47.38	-70.05	L1	Summer 2012	0
Ligne Pur Savage Invasive	Quebec	48.06	-69.29	M	Summer 2012	0
St. Mathieu Native	Quebec	48.19	-68.97	N	Summer 2012	0
Hebertville Native	Quebec	48.39	-71.67	N	Summer 2012	0
Galilee Invasive†	Rhode Island	41.38	-71.51	M	Winter 2013	69
Georgetown	South Carolina	33.36	-79.27	M	Summer 2012	0
Balmorhea	Texas	30.94	-103.79	I	Summer 2012	0
St. George	Utah	37.09	-113.57	N	Summer 2013	0
Clear Creek	Utah	38.58	-112.26	N	Summer 2012	0
Green River	Utah	40.16	-110.22	N	Summer 2012	0
Springhill Provo Bay	Utah	40.18	-111.64	N	Summer 2012	0
Utah Lake Park	Utah	40.24	-111.73	M	Summer 2012	0
I-80	Utah	40.77	-112.06	M	Summer 2012	0
Farmington	Utah	40.95	-111.93	M	Summer 2012	0
Tappahannock†	Virginia	37.92	-76.86	M	Winter 2013	44
Rappahannock Native 1*†	Virginia	38.07	-76.95	N	Summer 2012 + Winter 2013	148

(Table A.2 continued)

Site name	Country/State/ Province	Latitude	Longitude	Genotype	Sampling period	Galls collected
Rappahannock Native 2†	Virginia	38.07	-76.95	N	Winter 2013	32
Barnhart Drain Rd	Washington	46.29	-120.18	N	Summer 2013	0
Tappenish 1	Washington	46.31	-120.20	N	Summer 2013	0
Tappenish 2	Washington	46.32	-120.22	N	Summer 2013	0
Ellensburg	Washington	46.94	-120.51	N	Summer 2013	0
Winthrop Harbor	Wisconsin	42.48	-87.85	M	Summer 2013	0
Zion	Wisconsin	42.49	-87.91	M	Summer 2013	0
Pleasant Prairie Park	Wisconsin	42.54	-87.92	M	Summer 2013	0
Madison	Wisconsin	43.11	-89.32	M	Summer 2013	0
Quinta Do Lago	Portugal	37.05	-8.00	M	Summer 2012	0
Lagos 125	Portugal	37.12	-8.67	M	Summer 2012	0
Castro Marim	Portugal	37.21	7.43	M	Summer 2012	0
Pateira de Fermentelos 2	Portugal	40.58	-8.54	M	Summer 2012	0
Pateira Regeixo Park	Portugal	40.58	-8.53	M	Summer 2012	0
Rua Da Encarnacao	Portugal	40.60	-8.74	M	Summer 2012	0
Rue Du Pont Nuef 2	France	44.64	-1.01	M	Summer 2012	0
Ornitological Park 1	France	44.64	-1.02	M	Summer 2012	0
Huitres Banc	France	44.68	-1.02	M	Summer 2012	0
Briere Regional Park 2	France	47.36	-2.32	M	Summer 2012	0
St Joachim	France	47.39	-2.20	M	Summer 2012	0
La Roche Bernard	France	47.52	-2.30	M	Summer 2012	0
Bourgoyen House Trail	Belgium	51.07	3.67	M	Summer 2012	0
Scheldt Estuary 2	Belgium	51.34	4.19	M	Summer 2012	0
Scheldt Estuary	Belgium	51.35	4.23	M	Summer 2012	0
ENG	Denmark	55.21	10.25	M	Summer 2012	0
Stilling	Denmark	56.05	10.01	M	Summer 2012	0
Brabrand Lake	Denmark	56.14	10.12	M	Summer 2012	0
Melsomskogen Natursti	Norway	59.22	10.34	M	Summer 2012	0

Grevetien Ilene Reserve	Norway	59.28	10.40	M	Summer 2012	0
Semslinna	Norway	59.28	10.37	M	Summer 2012	0

RESULTS FROM ANCOVA INCLUDING PATCH SIZE AND STEM DENSITY

Table A.3. Results of analysis of covariance (ANCOVA) model testing the effects of *Phragmites australis* phylogeographic group (NA native, NA invasive, and EU native) on *Lipara* infestation level (logit transformed), and using patch size and stem density as covariates (n = 22).

Independent variable	**df**	***F*-value**	***P*-value**
Patch size	1, 17	0.305	0.5882
Stem density	1, 17	3.119	0.0953
Genotype/region	2, 17	15.397	**0.0001**

STUDIES REPORTING PARASITISM OF *LIPARA* IN EUROPE

Table A.4. List of studies reporting percent parasitism of *Lipara* in Europe. Each data point was taken directly from the literature or estimated from data or figures. The percent parasitism was estimated from at least one independent patch of *Phragmites australis*.

Location	*Lipara* species	Parasitism rate	Reference
Germany	*L. pullitarsis*	59	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	47	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	46	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	30	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	30	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	28	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	25	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	23	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	22	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	21	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	14	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	0	Athen and Tscharntke 1999
Germany	*L. pullitarsis*	2	Tscharntke 1994
Germany	*L. pullitarsis*	3	Abraham and Carstensen 1982
England	*L. rufitarsis*	15	Reader 2001
England	*L. rufitarsis*	26	Reader 2003
Germany	*L. rufitarsis*	19	Tscharntke 1994
Central Europe	*L. similis*	22	Schwarzlander and Hafliger 2000
Germany	*L. similis*	22	Tscharntke 1994

APPENDIX B. SUPPLEMENTARY MATERIAL FOR CHAPTER 3

PHRAGMITES AUSTRALIS POPULATIONS VISITED IN THE FIELD SURVEY

Table B.1. List of *Phragmites australis* field populations surveyed for the proportion of stems galled by *Lipara rufitarsis*.

Population location (ID code)	Latitude	Longitude	Lineage
Mackay Island, NC (NCN) †	36.51	-75.95	Native
Mackay Island, NC (NCN2)	36.52	-75.95	Native
Mackay Island, NC (NCM) †	36.52	-75.96	Invasive
Mackay Island, NC (NCM2)	36.52	-75.96	Invasive
Tappahannock, VA (VAM)	37.92	-76.86	Invasive
Rappahannock River, VA (VAN) †	38.07	-76.95	Native
Rappahannock River, VA (VAN2)	38.07	-76.95	Native
Choptank, MD (MDN) †	38.77	-75.97	Native
Choptank, MD (MDM) †	38.77	-75.97	Invasive
Estell Manor, NJ (NJN)	39.42	-74.73	Native
Estell Manor, NJ (NJM) †	39.41	-74.74	Invasive
Appoquinimink, DE (DEN) †	39.45	-75.65	Native
Appoquinimink, DE (DEM) †	39.45	-75.65	Invasive
Appoquinimink, DE (DEM2)	39.45	-75.65	Invasive
Pettipaug, CT (CTN)	41.36	-72.38	Native
Pettipaug, CT (CTM)	41.36	-72.38	Invasive
Pettipaug, CT (CTM2)	41.37	-72.38	Invasive
Galilee, RI (RIM) †	41.38	-71.51	Invasive
East Sandwich, MA (ESN)	41.74	-70.43	Native
East Sandwich, MA (ESM)	41.74	-70.43	Invasive
Webhannett, ME (MEN)	43.30	-70.58	Native
Webhannett, ME (MEM) †	43.30	-70.58	Invasive
Nonesuch, ME (NSN) †	43.58	-70.33	Native
Spurlink, ME (SLN) †	43.59	-70.25	Native
Sawyer Road, ME (REM) †	43.59	-70.26	Invasive

†Populations which were common to both the field and common garden studies.

PHRAGMITES AUSTRALIS POPULATIONS USED IN THE COMMON GARDEN EXPERIMENT

Table B.2. List of *Phragmites australis* populations used for the common garden experiment at the University of Rhode Island.

Population location (ID code)	Latitude	Longitude	Lineage
John Prince Park, FL (FLI)*	26.60	-80.08	Gulf
Savannah Preserve, FL (SPI)	27.52	-80.35	Gulf
McKee, FL (MKI)	27.61	-80.37	Gulf
Pass A Loutre, LA (PLM)	29.13	-89.23	Invasive
Pointe Aux Chenes, LA (PCI)	29.45	-90.46	Gulf
Rockefeller Road, LA (RRM)	29.69	-92.84	Invasive
Rockefeller Boat Launch, LA (RBI)	29.72	-92.77	Gulf
Rockefeller Wildlife Refuge, LA (RWI)	29.73	-92.83	Gulf
East Cameron, LA (ECM)	29.78	-93.29	Invasive
Intracoastal City, LA (ICI)	29.79	-92.20	Gulf
Santee Coast Guard, LA (SCI)	29.81	-90.33	Gulf
Creole, LA (CRI)	29.84	-93.11	Gulf
Creole, LA (CRM)	29.88	-93.08	Invasive
Victorville, CA (MRN)	34.54	-117.29	Native
I-40, AZ (AZM)	34.72	-114.49	Invasive
Salinas River, CA (SRN)	35.50	-120.65	Invasive
Mackay Island, NC (NCN) †	36.51	-75.95	Native
Mackay Island, NC (NCM) †	36.52	-75.96	Invasive
Rappahannock River, VA (RDM)	37.94	-76.83	Invasive
Rappahannock River, VA (RRN)	38.05	-76.93	Native
Rappahannock River, VA (VAN) †	38.07	-76.95	Native
Wimico Creek, MD (WCN)	38.28	-75.69	Native
Choptank, MD (MDN) †	38.77	-75.97	Native
Choptank, MD (MDM) †	38.77	-75.97	Invasive
Severn River, MD (SRM)	38.93	-76.51	Invasive
South River, MD (SOM)	39.07	-76.55	Invasive
St. Jones River, DE (SJN)	39.16	-75.46	Native
Estell Manor, NJ (NJM) †	39.41	-74.74	Invasive
Appoquinimink, DE (DEN) †	39.45	-75.65	Native
Appoquinimink, DE (DEM) †	39.45	-75.65	Invasive
Block Island, RI (BIM)	41.18	-71.57	Invasive
Block Island, RI (BIN)	41.18	-71.57	Native
Ragged Rock, CT (RAM)	41.31	-72.36	Invasive
Ragged Rock, CT (CTN)	41.31	-72.36	Native
Charlestown, RI (CHM)	41.36	-71.64	Invasive
Moonstone Beach, RI (MSM)	41.37	-71.57	Invasive

(Table B.2 continued)

Population location (ID code)	Latitude	Longitude	Lineage
Galilee, RI (RIM) †	41.38	-71.51	Invasive
Naushon Island, MA (NFM)	41.47	-70.76	Invasive
Naushon Island, MA (NFN)	41.47	-70.76	Native
Falmouth, MA (FPM)	41.59	-70.64	Invasive
Falmouth, MA (FPN)	41.59	-70.64	Native
Humboldt, NV (NVN)	41.59	-118.55	Native
Warren, RI (JPM)	41.71	-71.29	Invasive
Bristol Audubon Society, RI (BAM)	41.71	-71.29	Invasive
Bristol Audubon Society, RI (BAN)	41.71	-71.29	Native
Warren, RI (JPN)	41.71	-71.29	Native
Herring River, MA (MAM)	41.94	-70.06	Invasive
Agawam Lake, MA (GLM)	42.26	-73.33	Invasive
Montezuma, NY (NYM)	42.94	-76.74	Invasive
Montezuma, NY (NYN)	42.94	-76.74	Native
Great Bay, NH (GBM)	43.05	-70.90	Invasive
Great Bay, NH (GBN)	43.05	-70.90	Native
Webhannett, ME (MEM) †	43.30	-70.58	Invasive
Rachael Carson, ME (RCM)	43.32	-70.57	Invasive
Rachael Carson, ME (RCN)	43.32	-70.57	Native
Libby River, ME (LRM)	43.58	-70.33	Invasive
Nonesuch, ME (NSN) †	43.58	-70.33	Native
Spurlink, ME (SLN) †	43.59	-70.25	Native
Sawyer Road, ME (REM) †	43.59	-70.26	Invasive
Nonesuch River, ME (NRN)	43.62	-70.33	Native
Yarmouth, ME (YMM)	43.80	-70.17	Invasive
Holt Research Forest, ME (MEN)	43.87	-69.78	Native
New Meadows River, ME (MRM)	43.90	-69.89	Invasive
Bath, ME (BCM)	43.91	-69.83	Invasive
Pierce Hill Road, ME (PHM)*	45.08	-69.91	Invasive
Lac St. Francois, Quebec (SFN)*	45.88	-71.12	Native
Moncton, New Brunswick (NBM)	46.07	-64.72	Invasive
Moncton, New Brunswick (NBN)	46.07	-64.72	Native

†Populations which were common to both the field and common garden studies.

*Populations which were excluded from the final model as outliers based on quantile-quantile plots and Cook's D.

RESULTS FROM GENERAL LINEAR MODELS FOR EFFECTS ON *LIPARA* HERBIVORY

Table B.3. Results from general linear model analyses for the effects of latitude, latitude^2 and stem characteristics during the *Lipara rufitarsis* oviposition period on the proportion of stems galled of native, invasive, and Gulf lineages of *Phragmites australis* in the common garden experiment. Analyses were separated by lineage and tests were only performed for variables which were significant as main or interaction effects in the AICc best model (which was all variables). Statistically significant gradients ($P < 0.05$) are in bold, and we report whether the *P. australis* lineages had non-parallel (lineage interaction in AICc best model) or parallel (no lineage interaction) relationships for each stem characteristic. Goodness of fit is reported as 1-(residual deviance/null deviance) (Menard 2000).

	Native			**Invasive**			**Gulf**			
Independent variable	**Slope (± S.E.)**	***R*^{2}**	***P***	**Slope**	***R*^{2}**	***P***	**Slope**	***R*^{2}**	***P***	**Gradient**
Latitude	**0.070 ± 0.020**	**0.054**	**<0.001**	0.010 ± 0.008	0.004	0.218	**0.589 ± 0.252**	**0.224**	**0.019**	Non-parallel
Latitude^2	**0.001 ± 0.0002**	**0.050**	**<0.001**	0.0001 ± 0.0001	0.002	0.384	**0.010 ± 0.004**	**0.224**	**0.019**	Non-parallel
Stem density	**0.074 ± 0.014**	**0.115**	**<0.001**	0.021 ± 0.014	0.006	0.131	**-0.214 ± 0.068**	**0.296**	**0.002**	Non-parallel
Stem diameter	**0.301 ± 0.089**	**0.049**	**0.001**	**0.215 ± 0.057**	**0.039**	**<0.001**	**0.699 ± 0.199**	**0.398**	**<0.001**	Non-parallel
Stem height	**-0.021 ± 0.004**	**0.143**	**<0.001**	**-0.031 ± 0.003**	**0.484**	**<0.001**	-0.001 ± 0.008	0.0003	0.910	Parallel

APPENDIX C. SUPPLEMENTARY MATERIAL FOR CHAPTER 4

RESULTS FROM AICC MODEL SELECTION FOR *PHRAGMITES AUSTRALIS* AND *SPARTINA ALTERNIFLORA*

Table C.1. AICc best models (ΔAICc ≤ 2) to explain variation in total biomass produced per day and proportion of biomass allocation to belowground tissues for each plant species (*Phragmites australis* or *Spartina alterniflora*). Explanatory variables: L = *P. australis* lineage (native, European, Gulf), C = presence/absence of an interspecific competitor, N = high/low nutrient availability, and S = live/sterile soil inoculum. × denotes interactions between explanatory variables.

Dependent variables	Models	AICc	ΔAICc	AICc weight
Phragmites australis				
Total biomass produced (per day)	C N S	-863.9	0.00	0.436
	C N S C×S	-862.4	1.48	0.207
	C N S N×S	-862.2	1.66	0.190
	C N S C×N	-862.0	1.92	0.167
Proportion of biomass allocated to belowground tissues	C L N S L×N	-1222.0	0.00	0.436
	C L N L×N	-1220.4	1.63	0.193
	C L N S C×S L×N	-1220.4	1.65	0.192
	C L N S C×N L×N	-1220.3	1.78	0.179
Spartina alterniflora				
Total biomass produced (per day)	C L N S C×N C×S L×N L×S	-1245.2	0.00	0.711
	C L N S C×N C×S L×N L×S N×S C×N×S	-1243.4	1.80	0.289
Proportion of biomass allocated to belowground tissues	C L N S C×N L×S	-1153.6	0.00	0.329
	C L N S C×N C×S L×S	-1153.0	0.56	0.249
	C L N S L×S	-1152.3	1.33	0.169
	C L N S C×S L×S	-1151.7	1.86	0.130
	C L N S C×N L×S N×S	-1151.6	1.99	0.122

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VITA

Warwick Allen was born and raised in Christchurch, New Zealand, and has been interested in the natural world since he can remember. This interest was fostered by a childhood where every free moment was spent hiking, mountain biking, kayaking, skiing, and camping in the great outdoors with his family. His passion for ecological research and conservation was formalized during his undergraduate degree at Lincoln University, New Zealand, stimulated by interesting courses, field trips, and summer research programs, working on a range of projects and systems, including penguins, plants, and insects. After completing his undergraduate degree, he worked as a technician for 18 months at Plant and Food Research on projects using chemical ecology to improve the detection and control of crop pests. In August 2011, Warwick joined the Cronin lab at Louisiana State University. For his dissertation, he studied the role of biogeographic variation in multitrophic interactions in the invasion success of the cosmopolitan wetland grass species, *Phragmites australis*. In September 2016, Warwick will return to New Zealand and Lincoln University to begin a postdoctoral fellowship position researching how traits of ecological interaction networks can be used to predict the success or failure of introduced species. Warwick ultimately hopes that his past and future research will make a valuable contribution to the understanding and conservation of natural systems on our beautiful planet.